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PRIMARY MALIGNANT HEMANGIOMA OF THE SPLEEN WITH MULTIPLE LIVER METASTASES *

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Malignant tumors of the blood vascular system which metastasize to other parts of the body via the blood stream, are very rare. Less than a dozen such proved cases have been reported. Benign newgrowths, on the other hand, are not uncommon and the literature abounds with descriptions of them and discussions of their origin and growth. These non-malignant neoplasms of the blood vessels may be localized and definitely circumscribed, or they may progress slowly, exhibiting a considerable amount of local proliferation and invasion. By Ewing [1] they are classed as simple hemangiomata (hemangioma simplex), of which he recognizes several types. The most important, in his opinion, are (1) the common, localized, non-progressive nevus vinosus of the skin, usually congenital in origin; (2) the subcutaneous plexiform angioma of young children, in which there may be considerable local invasion of the underlying structures; and (3) the more hyperplastic type of growth called by Ziegler the hemangioma hypertrophicum, in which the tumor cells grow quite rapidly and often show poor cellular differentiation.

These tumors may be further characterized as being either capillary or cavernous in type, according as the vessels of the growth have minute, capillary-like lumens, or appear as large distended spaces separated from each other by walls which are quite thin. Occasionally in these newgrowths vessel lumens are entirely absent,

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as in certain of the so-called endotheliomata, and the tumor cells grow in whorls or solid masses. By and large, however, benign angiomata consist of single or multiple circumscribed nodules in which small or large new-formed vascular channels are present.

The commoner forms of such angiomata appear on the skin or in the underlying subcutaneous layers. The face, scalp, labia, scrotum, extremities, and axillae are the regions chiefly involved. But the deeper tissues and organs may be affected as well. The majority of the growths are circumscribed and encapsulated, although not infrequently some of them evidence a certain local proliferation by the invasion and infiltration of structures in the neighborhood of the original growth. Cases of such local malignancy are on record, Hildebrand,[2] Gascoyen,[3] Cruveilhier.[4] Often these locally invasive neoplasms have led to the death of the patient.

A malignant hemorrhagic newgrowth of the skin of elderly subjects, first described by Kaposi[5] in 1872, is also characterized by the formation of new blood vessels. In their initial stages the lesions of this disease are inflammatory and not neoplastic. They exhibit a definite relation to certain predisposing factors such as vascular disturbances, sclerosis, trauma, alcoholism, etc. Later, however, they may become true tumors, the malignant cells infiltrating the surrounding tissues and occasionally metastasizing to the viscera. These tumors, according to Pick,[6] consist of new, dilated lymph and blood vessels about which the stroma is infiltrated with chronic inflammatory cells. Endothelial proliferation is prominent and undifferentiated sarcomatous cells are present in the perivascular tissues. Such malignant nodules are generally hemorrhagic, often pigmented, and may break down and ulcerate.

The nature of this disease is obscure. Some consider it to be an infectious granuloma of unknown etiology which, in its later stages, takes on genuine neoplastic properties. Others, however, believe the condition to be a tumor from the start, namely, a metastasizing capillary angioma which tends to undergo sclerosis. Vasoformative cells are certainly present, but because of its association with a chronic inflammatory lesion, its definite relationship to predisposing causes, and its tendency to ulcerate, this malignant growth differs from the primary blood vessel tumors about to be described.

True vascular newgrowths which exhibit the peculiar characteristic of malignancy, that of giving rise to the formation of metastases

in other parts of the body, are rare. Several cases, however, are on record. One of the most striking of these is reported by Borrmann.[7] His patient, a young woman of twenty-six, had, in the skin of the right breast, an angiomatous growth which recurred repeatedly after operative removal. Ultimately the tumor led to the death of the patient by the formation in the lungs of multiple metastatic nodules of the same type. In this case the primary tumor was structurally a simple angioma, histologically non-malignant. The secondary nodules, however, exhibited somewhat greater cellular activity, but nowhere did the neoplasm have the microscopic characteristics of a malignant growth.

Ewing observed a similar case in which a primary hemangiomatous tumor appeared in the left breast of a female patient. This was followed by other tumors of the same nature in the skin and mucous membranes. There was evidence also of pulmonary involvement.

Shennan [8] described the remarkable case of a woman of twenty-three whose history for six years had been marked by mediastinal pressure, hemoptysis, enlargement of the spleen, hemorrhage into the pleural cavities, and the formation of vascular nevi in the skin. At necropsy there were found multiple cavernous hemangiomata which involved the entire spleen, the thymus, the mediastinal lymph nodes, and portions of the lungs, liver, and bone marrow. Histologically the tumors were not malignant in appearance. It was felt, however, that the neoplasm originated in the mediastinum and that the other growths were undoubtedly of metastatic origin. Locally these tumors exhibited a certain invasive tendency.

Homans [9] published a short account of the case of a young woman of twenty-two who was very anemic and whose abdomen had been enlarged for over a year. On several occasions bloody fluid had been removed from the peritoneal cavity. Laparotomy revealed a large abdominal mass of which a part was excised. Histologically it was an angiomatous tumor. Later the spleen was removed. It was infiltrated with multiple angiomata. The tumor was considered to be primary in the spleen with metastases to the other situations.

Langhans [10] presented the case of a thirty-year-old man in whose left upper abdomen there was a large pulsating tumor. The mass grew rapidly larger and the patient died about two months after the onset of symptoms. On postmortem examination the spleen was found to be enormously enlarged and adherent to the diaphragm.

On section only a narrow zone of relatively normal splenic tissue could be recognized at the periphery of the organ. Histologically lymph nodules were not visible; trabeculae were present unchanged; the pulp was greatly altered. The remainder of the organ consisted of a firm fibrous stroma in which there were numerous blood channels containing brownish clots. Microscopically this part of the spleen was identified as a cavernous hemangioma in which endothelial cells were seen to be proliferating rapidly.

In the greatly enlarged liver there were many similar blood-filled tumor nodules which almost entirely replaced the normal parenchyma. Histologically these were definite angiomatous tumors of the cavernous type. In Langhans' opinion the spleen was undoubtedly the organ affected primarily and the nodules of the liver were metastatic in nature.

Theile [11] described four cases of angioma of the spleen which were collected out of about nineteen hundred necropsies done over a period of four and a half years in the laboratory of Professor Lubarsch. Three of these were non-malignant and are mentioned very briefly. The fourth, because of its malignant characteristics, is worth reviewing carefully.

The patient was a fifty-six-year-old man in whose abdomen a large tumor could be palpated. This proved to be a greatly enlarged spleen which was removed surgically. The patient died the day after operation from internal hemorrhage. At necropsy the important additional findings were multiple newgrowths of a metastatic nature in the liver, lungs and stomach.

Macroscopically the spleen was very large, weighing 2500 gm. The capsule was thickened and covered with blood clots. On section little of the normal tissue could be recognized. Numerous whitish tumor nodules were present on the dark reddish cut surfaces. In places many large blood-filled spaces were seen. Histologically there were two types of growth, one in which the tumor consisted definitely of small and large vessels, the other where the atypical cells took on a sarcomatous character and grew rapidly, without great differentiation.

The liver contained many tumor nodules which resembled the splenic lesions. Microscopically some of these consisted of thin-walled capillaries in a fibrous stroma; others were more cellular, resembling the sarcomatous foci in the spleen.

Metastatic nodules were found also in the lungs and in the stomach. The pulmonary lesions resembled those in the liver, but the nodules in the stomach wall were sarcomatous in structure. No pure angiomata were found.

Theile considers these two types of growth to be but phases in the development of a single tumor of angioblastic origin. The differences observed histologically he believes to be anaplastic in nature. The tumor, therefore, he designates a "sarcomatous angioma" of the spleen which gave rise to multiple metastases.

Finally Jores [12] described a somewhat similar case of sarcomatous angioma of the spleen in which the organ was found to be much increased in size, about 3600 gm., and almost entirely necrotic. The liver was likewise enlarged, weighing 8600 gm. Both organs were infiltrated with angiomatous tumors which in some places were histologically benign, in others of sarcomatous nature. The primary tumor Jores considers to have been in the spleen, the liver involvement being secondary.

In these seven reports the angiomatous tumors which are described exhibit a common characteristic, namely, the formation of metastatic nodules of similar histologic structure in distant parts of the body. In four cases the tumors were histologically benign, but in those of Langhans, Theile, and Jores the newgrowths were definitely of malignant nature. Such tumors of blood vascular origin, however, are rare.

Report of Case

There came recently to the Boston City Hospital a patient whose history and clinical course presented a difficult diagnostic problem. At necropsy a large, blood-filled tumor of the spleen was found. Numerous neoplastic nodules were scattered throughout the splenic pulp and the enlarged liver was the seat of great numbers of secondary growths. Histologically the tumor proved to be a rapidly growing hemangioma of the spleen with multiple liver metastases. Because of its unusual features the case is here reported in detail:

History and Clinical Course: J. R., a white American laborer 25 years of age, was admitted to the Boston City Hospital December 12, 1924, complaining of pain in the back and swelling of the abdomen. His past history was unimportant. The present illness began about four weeks before admission when the patient received a sharp blow in the abdomen from a small hand truck which he

was pushing. For several days thereafter he had attacks of rather marked abdominal pain. The pain was sharp and knife-like, was situated in the loins and lower back, and radiated upward to the shoulders. Ten days before admission his abdomen began to swell. His skin was transiently yellow and at these times the stools were pale and the urine high-colored. The patient became progressively weaker, his appetite failed, and he lost about four pounds in weight.

The patient seemed to be in considerable distress, complaining constantly of pain in the lower back. The skin was pale but not icteric. The chest appeared symmetrical, with moderate fullness at the junction of lower chest and abdomen. The abdomen was distended. Over the epigastrium the percussion note was flat and slight tenderness was elicited on palpation. The liver edge could not be felt. Ascites was not noted.

At the time of admission his blood pressure was systolic 140 and diastolic 100; temperature 98.4° F; pulse 72; respiration 28. The red cell count was 5,280,000 per cmm. Leukocytes 14,300. Hemoglobin 75 per cent (Sahli). A blood smear stained with Wright's stain showed normal cells and a normal differential count.

The urinary examination was negative except for the presence of bile in moderate amounts. The stools were clay-colored. On the third day after admission the skin and sclerae were slightly icteric. Although the liver could not be definitely palpated it was felt that the fullness in the upper abdomen was due to enlargement of that organ. At this time ascites was found to be present.

Paracentesis resulted in the removal of 2150 cc. of slightly cloudy, bile-stained fluid which was transudative in character and sterile on culture. After the tap the mass in the upper abdomen was recognized definitely as a greatly enlarged liver. The spleen could not be felt. The removal of the fluid, however, brought no relief and the patient became more restless and the pain more severe. Two days later the abdomen was again full of fluid. The temperature gradually fell until it was subnormal. The pulse became more rapid and weak. The red count dropped to 2,728,000 per cmm., with hemoglobin of 60 per cent (Sahli).

A surgical consultant advised immediate laparotomy. After a preliminary transfusion of 600 cc. of citrated blood the abdomen was opened and the peritoneal cavity was found to be full of fresh and partly clotted blood. No ruptured viscus could be discovered. The spleen and liver were greatly enlarged and the surface of the latter organ was studded with soft, dark reddish nodules of varying size. The kidneys were apparently negative. A diagnosis of carcinoma of spleen and liver was made and the incision closed. In spite of active supportive treatment the patient's temperature remained subnormal and he sank gradually into unconsciousness, dying about sixty hours after the operation.

Necropsy Protocol

The body is that of a well developed adult white male. Nothing of note is found on superficial examination.

Peritoneal Cavity: The peritoneal cavity contains approximately 1000 cc. of dark reddish fluid in which there is a considerable amount of clotted blood. The serous surfaces, both parietal and visceral, are

inflamed and are covered with fresh fibrin, which mats together the coils of small intestine and binds the omentum closely to the surgical incision in the peritoneum. The liver is greatly enlarged and extends down to a point 11 cm. below the ensiform process and 8 cm. below the costal margin in the right mammary line. Its surface is seen to be studded with numerous soft, bluish red nodules which will be described more at length later. On pulling the stomach and the splenic flexure of the colon toward the midline, a large amount of clotted blood, much of which is old and friable, is found beneath these structures. Removal of these clots discloses the greatly enlarged spleen, to which they are adherent. This organ is situated normally but it is approximately four or five times its usual size. In many places its thickened capsule is firmly attached by fibrous tissue to the diaphragm and portions of neighboring organs. With difficulty these adhesions are divided and the organ removed.

Spleen: Weight 520 gm. The organ measures 15 by 5 by 8 cm. in size exclusive of a large, somewhat spherical tumor mass which is firmly attached to its diaphragmatic and gastric surfaces. This mass measures 10 by 9 by 9 cm. and its pedicle-like base at the point of origin in the spleen is approximately 7 cm. in diameter. It is quite definitely a large primary tumor of the spleen itself. The surface of the organ, exclusive of the tumor mass, is bluish red in color and is thrown into small ridges which give it a corrugated appearance. Here and there stringy fragments of soft clotted blood are adherent to the thickened capsule, and in many places there project above the capsular surfaces small, rounded, dark reddish nodules which are quite soft and collapsible. The shape of the organ, however, except for the projecting tumor mass, is well preserved.

The large spherical tumor is covered with old, granular blood clots beneath which is the thick, tough, yellowish capsule. In one place where the clotted blood is especially dense, there is an irregular capsular tear which measures 1.5 cm. in length. This opening is definitely of ante mortem origin, for its edges are covered with fibrin which is undergoing organization. On sectioning the mass the knife passes through the tough fibrous capsule and plunges into a series of large cavities which are filled with soft, dark reddish, necrotic, bloody material. In the center of the tumor and extending down into its pedicle, there is a core of yellowish white, firm tissue which measures 1.5 cm. in diameter. Imbedded in this tissue there is a

blood vessel filled with a long, friable thrombus which in many places is adherent to the vessel wall. The entire lumen of the vessel, however, is not occluded.

On section of the spleen the tumor mass is found to be completely encapsulated so that it is separated from the splenic tissue. It now appears to be but one of many tumors of this organ. The others are smaller and less distinct, and show little or no necrosis. They are scattered throughout the spleen appearing often immediately beneath the capsule, above which some of them project. The majority of the nodules, however, are present in the deeper tissues. In general these lesions are irregular, somewhat spherical cavities which vary considerably in size, the smallest visible ones measuring about 0.1 cm. in diameter, the largest approximately 1 cm. The latter are trabeculated, for thin, yellowish white strands of connective tissue are seen projecting into them or dividing them into smaller spaces. The most noteworthy characteristic of all of the nodules is that they contain considerable quantities of blood which gives them a deep reddish brown color and thus sharply demarcates them from the surrounding tissues. It is impossible to recognize the normal architecture of the spleen and between the individual lesions there exists a stroma of reddish, moist tissue which in places is markedly sclerosed. The nature of these hemorrhagic nodules is not at once evident. For the moment they are considered to be newgrowths of unknown type.

Liver: Weight 4250 gm. The organ is much enlarged. The capsular surface is moist and glistening but is studded with raised, rounded nodules which resemble the superficial tumors of the spleen. Most of these are soft and collapsible and appear dark bluish red in color. They vary greatly in size. Between them the liver capsule has a greenish brown hue with faint yellowish mottling. On sectioning the organ the entire parenchyma is found to be infiltrated with nodules similar to those seen on the surface. In shape these structures are irregularly spherical, varying in size from 0.5 cm. to 3 or 4 cm. in diameter. The largest lesions consist of trabeculated spaces filled with clotted blood. The smallest are yellowish and contain less blood. All of the nodules are clean-cut and distinct. The tissue of which they are composed is yellowish white and quite friable. Between these neoplastic growths, which are present everywhere in the liver, the parenchymal tissue is pale greenish brown in color and

appears markedly atrophic. A faint yellowish mottling, confined to the central portions of the lobules is noted.

Aside from slight congestion of the lungs and moderate scarring at their apices, the examination of the remaining organs shows no noteworthy changes.

Microscopic Examination

Spleen: In sections from the spleen the normal reticular tissue of the pulp is recognized only in occasional areas. Small, unaltered lymph nodules are rarely visible and most of the architecture of the organ is either destroyed or obliterated by an extensive neoplastic growth which invades and infiltrates the entire organ.

Microscopically this newgrowth is characterized by the formation of narrow channels and dilated cavities which are filled with blood. In some places these newly formed spaces are lined with atypical cells which grow rapidly and profusely, exhibiting the characteristics of malignant cells. In others the neoplastic nature of the growth is less evident and the numerous blood vessels are imbedded within a fibrous stroma which varies in amount and density in different regions. Those parts of the neoplasm which are least progressive resemble vascular scars. There is thus evident a wide variation in histologic structure. In all places, however, the tumor exhibits one common and outstanding feature, the formation of blood-containing channels.

The most characteristic portions of the neoplasm are those in which the tumor cells are growing rapidly and appear most atypical and undifferentiated. In such regions there are formed large abnormal spaces which are filled with blood. Most of these spaces are irregularly spherical in shape and measure from 0.5 to 2 cm. in diameter. Into them there project varying numbers of papillary processes on which the typical tumor cells grow profusely. These processes consist of thin, pink-staining strands of collagen in which no blood vessels or other structures are demonstrable, except rarely when the nucleus of a connective tissue cell or the lumen of a small capillary is seen. Such processes sometimes project inward from the wall to form a complex maze within the space (Fig. 2); sometimes they radiate from a vascular fibrous core which occupies a central position in the cavity. About this core the papillary structures are gathered in concentrically arranged strands.

It is readily seen that these projections are not simple processes of stroma which merely grow out as short, finger-like extensions into the adjacent space. They are long and delicate, and branch in many directions, forming an intricate maze of tortuous collagenous strands. Because of their profuse arborization they are cut in every possible plane so that they vary in shape from narrow strips to isolated circular or oval fragments which lie free in the blood-filled spaces. They thus resemble in many ways sections through placental villi.

Attached to the papillary projections in multicellular layers are the typical tumor cells. These are large, irregular in shape, and are so crowded together that they maintain their attachment to the underlying stroma by nothing more than delicate strands of cytoplasm (Fig. 7). They have thus an irregular, piled-up arrangement, in places resembling the structure of pseudo-stratified epithelium. The cytoplasm of these cells is scanty. Where it is seen it appears pale bluish in color, without specific structure. The nuclei are large and vesicular, often filling the entire cell. They contain small amounts of chromatin material in the form of minute granules scattered irregularly throughout a faintly reticulated nucleoplasm. Nucleoli are not present. Proliferation of these cells is rapid as is evidenced by the presence of numerous mitotic figures, as many as six or eight being found in a single oil-immersion field (Fig. 8).

Within the papillary-lined spaces, often closely surrounding the papillary structures themselves, are varying numbers of red blood cells. In some of the nodules they are present in great abundance, giving a deep red color to these lesions so that they stand out prominently even on naked-eye examination of the section. In other places, however, the blood cells are less numerous. Rarely, small fibrinous clots are found within the interpapillary spaces. Some of these are true thrombi, for there is injury and even necrosis of the adjoining tumor cells. Most of the clots, however, are of postmortem character.

Rapidly growing tumor nodules such as these just described are found throughout the spleen. Although they vary considerably in size, they exhibit everywhere the formation of dilated spaces which contain papillary projections in varying numbers and in different degrees of complexity. In a striking way some of the smaller lesions resemble a typical coccidial cyst of the liver in which projections of epithelium are characteristically formed. The blood spaces them-

selves may be seen to communicate directly with readily recognized blood vessels, and the atypical tumor cells which line them form a layer which is continuous with the normal endothelium of these vessels. Such abnormal spaces are seen, therefore, to be extensions of the normal vascular system.

The regions where tumor proliferation is least active are just as numerous as those where growth is rapid and invasive, but are less striking because of their resemblance to vascular scar tissue. In these situations sclerosis is marked and the blood vessels are small and well formed. The endothelial cells are less numerous, more highly differentiated, and only rarely exhibit active cell division. In general they resemble normal endothelium. In some places they are adjacent to or even surround rapidly growing nodules (Fig. 3). Foci of progressive infiltrative growth are thus often associated with regions of less marked neoplastic activity.

Between such extremes of tumor growth as have been described there are many other regions in which varying degrees of moderate neoplastic activity are evident. Here the atypical cells infiltrate the splenic tissue, adapting themselves to preëxisting stroma to form new blood vessels which are lined with large cuboidal cells. Such vessels are larger than the normal veins of the splenic pulp. They are usually numerous, being separated from each other by thin, fibrous walls. Not infrequently the tumor cells are found in the process of mitotic division.

The tumor, therefore, is of variegated histologic structure. In some places the growth is active, invasive, and malignant, and exhibits a tendency to form papillary projections within dilated spaces; in others there are diminishing degrees of endothelial proliferation, with a fibrous stroma which varies in amount and density inversely with the tumor activity. The neoplasm appears to have existed for a long time — months to years — for sclerosed, quiescent foci are present in most of the sections. But the rapidly growing, atypical tumor nodules are so abundant that one must consider the spleen to be now the seat of a highly malignant and progressive newgrowth in which blood vessel formation is the outstanding feature.

Sections from the large tumor at the upper pole of the spleen are disappointing. They show little of value histologically. The entire structure is infarcted and the tissues necrotic. Thick, degenerating bands of connective tissue divide the structure into smaller cavities

which contain large thrombi. Within the fibrous trabeculae there are numerous small blood vessels. In places there is evidence of papillary formation in some of the large spaces.

Liver: The lesions in the liver resemble those in the spleen. They are distributed throughout the organ but are most numerous in the neighborhood of the periportal spaces. Often the tumor completely surrounds these latter structures, infiltrating the adjacent parenchyma and causing atrophy and disappearance of the liver cells.

In the rapidly growing nodules the papillary processes are often more distinctly outlined than they are in the spleen and they present a less confusing picture. Here their tortuous, branching form is most clear and their arborizing character most evident (Fig. 2). In many of the nodules the processes originate from one portion of the wall of the space rather than from all parts equally.

Here and there in regions of less active growth there are large numbers of well formed blood vessels. In general these vessels are small, but not infrequently they reach the size of the channels which one finds in the common benign hemangioma (cavernoma) of the liver (Fig. 1). The endothelial cells which line these vessels are sometimes flat, sometimes cuboidal. They are more numerous, more primitive in appearance than are the endothelial cells which are found in normal blood vessels. It is possible to find a few mitotic figures in these cells so that they must be considered as actively proliferating, though not in the same profusion as those which are present in the more characteristic lesions where papillary processes are formed. In many places the tumor is sclerosed and resembles a vascular scar (Figs. 5 and 6).

The newgrowth everywhere infiltrates the liver tissue and atrophied parenchymal cells are readily demonstrated. Scattered throughout the connective tissue of the older lesions there are portions of bile ducts which stand out clearly from the fibrous stroma. Often the ducts are relatively increased in number, and in such areas, because of the absence of liver cells, the microscopic picture resembles in a striking manner that of acute yellow atrophy of the liver.

In several of these sections fragments of papillary processes carrying numbers of malignant cells are seen within preëxisting veins. In one such fragment there are two mitotic figures.

Careful histologic study of the remaining organs shows nothing of

note except for the presence of many endothelial leukocytes within the capillaries of the glomerular tufts.

The complete anatomical diagnoses are: Malignant hemangioma of the spleen with multiple metastases to the liver; infarct and rupture of large hemangioma of spleen; hemoperitoneum; acute fibrinous peritonitis; acute intracapillary glomerulonephritis, slight; healed pleuritis; healed tuberculosis of the lungs, apical; recent surgical incision of the abdomen; recent surgical incision of the antecubital fossa (site of transfusion).

Discussion

The tumor which has just been described has three outstanding characteristics. First, it reproduces blood vessels and blood-containing spaces. Second, it grows rapidly and invasively. Third, it gives rise to the formation of secondary nodules in another part of the body. It is, therefore, a malignant, metastasizing hemangioma and falls into a group of very rare neoplasms of the blood vascular system. It originated apparently in the spleen, for in this organ the oldest and most sclerosed lesions are found. The liver became secondarily involved and at the time of death was extensively infiltrated with the growth. From a histologic standpoint this new-growth differs in many respects from the metastasizing hemangiomata which have already been reviewed.

The tumor described by Theile in some places exhibited the characteristics of a slowly growing hemangioma, while in others its growth was more malignant and invasive, the neoplastic cells forming cords or sheets without definite structure. From the foregoing description it will be noted that a somewhat similar condition exists in the present case. Neoplastic growth in some situations is rapid and malignant; in others it is diminished, and the tumor resembles a typical benign hemangioma. Where cell proliferation is most active, however, the endothelial cells grow not in undifferentiated groups or masses, such as Theile described, but as papillary projections which extend into dilated, blood-filled spaces, much as epithelial cells grow in a papillary adenocystoma of the ovary. The tumor cells are multiplying so rapidly that there is not available sufficient space for them to be laid down in a single layer. They thus grow in several layers, often buckling or projecting inward

from the surface and carrying with them small amounts of delicate fibrous stroma, to form the characteristic papillary projections. Thus the malignant cells here tend to line large, blood-filled spaces or to project into them so that they are always in contact with the blood stream. These cells, therefore, while they are still abnormal and grow in atypical fashion, are less primitive and undifferentiated than are those in the more malignant parts of Theile's tumor. In the more slowly growing regions the two neoplasms are similar.

In some ways the papillary processes of this tumor resemble the "Kolben" described by Borrmann. In his preparations, however, such projections were short and knob-like and the endothelial cells which covered them were flat and well differentiated, forming a thin, single-layered membrane. They were in no sense malignant. Between these "knobs" there were narrow, irregular blood spaces which were really out-pocketings from a larger vascular cavity. Such narrow spaces Borrmann called "blood vessels of the second order," and in his opinion actual circulation through them was very sluggish, a fact which he felt accounted for the presence of thrombi which he found not infrequently in these situations.

In the present case, on the other hand, the papillary structures are long and delicate and the spaces corresponding to Borrmann's blood vessels of the second order, are narrow and branching. Thrombi, however, are only rarely found. Most of the cells of the tumor thus obtain adequate nourishment by direct imbibition and they are generally healthy and active. In the few places where small thrombi are present, there is evidence of degeneration of the tumor cells in the immediate neighborhood of the thrombus. This probably indicates that the circulation through some of the abnormal spaces was not sufficient for complete nourishment of the tumor tissue. Thrombosis may thus be explained, as in Borrmann's case, on the basis of previous degeneration of the adjoining cavity wall due to inadequate blood supply. There is no evidence of ante mortem clotting from any other cause.

The type cell of the tumor is the endothelial cell, since blood vessel formation is the outstanding characteristic of the newgrowth. The blood circulates freely through the newly formed channels and comes into intimate contact with the neoplastic cells. In many places the tumor cells are poorly differentiated and little can be made out cytologically. But within many of the malignant nodules there is

a definite gradation from undifferentiated to differentiated cells so that one is led to conclude that the tumor is a primary angioma in which both abnormal and relatively normal endothelial cells may be found.

True angioblasts, from a histologic standpoint, are not found in sections from the tumor. According to Sabin,[13] the characteristics of angioblasts are distinct enough to allow of their identification in sections. These cells, in the embryo, develop in solid or syncytial masses in which cell outlines can not be discerned. Later, by vacuolization or liquefaction of some of the cells in the syncytium, vesicles are formed, the walls of which become endothelium. There is a real differentiation of the cells thus left along the edges of the new formed vessels and the endothelial cell differs considerably from the angioblast, having a slightly longer nucleus and less granular cytoplasm. Careful study of the sections from the tumor here described fails to reveal any cells or cell groups which can be definitely identified as true "angioblasts." The neoplastic cells do not form syncytial masses. They tend to line spaces or cavities. True, they are not characteristic endothelial cells, but they appear to be less primitive than the embryonic angioblast. They are, in the writer's opinion, endothelial cells which have undergone neoplastic change and now grow wildly and profusely, penetrating and invading normal tissues or adapting themselves to the stroma of preformed blood vessels. They produce new, abnormal blood channels apparently by the method of sprouting, that is, by uncontrolled division and increase in the cytoplasm of the preëxisting tumor cells. This activity of the neoplastic cell thus resembles that of the mature endothelial cell which, as is well known, produces new cells by its own division, not by the addition of new, immature mesenchymal cells to the capillary wall. The evidence thus leads to the conclusion that the rapidly growing, atypical cells of this tumor originated not from primitive angioblasts, but rather from true endothelial cells. In their neoplastic state they reproduce endothelial structures.

Rapid proliferation of these neoplastic cells is evidenced by the presence of numerous mitotic figures (Fig. 8). Growth is most active in those regions where the papillary projections are conspicuous, for here the tumor cells are piled up on each other in irregular and atypical fashion. It is reasonable to assume that the rapid and uncontrolled growth of the cells in these regions, associated with

the occasional finding, in preëxisting vessels, of portions of papillae containing viable tumor cells, is indicative of malignancy and potential secondary tumor formation. The explanation for metastatic lesions is, then, not hard to discover. One wonders why the tumor is not more widespread. Perhaps such fragments of malignant tissue as were observed in some of the normal veins never reached the general circulation, being caught in narrow capillaries or sinusoids of the liver. Or it may be, as Handley [14] maintains in the case of mammary carcinoma, that there are antagonistic influences exerted within the blood stream upon the tumor cells which gain access to it. But it would be astonishing should such antagonistic forces be operative in the case of endothelial cells, since in the blood stream these cells still remain bathed in their normal nutritive fluid. Our ignorance of the nature of the subtle changes which take place in tissues and fluids is still very great, and a discussion of the factors which either favor or prevent metastasis formation would be most speculative. Certainly there are metastatic tumor growths within the liver and none elsewhere. So much is fact. The forces which determine this distribution are unknown.

In the case under discussion there are not only regions of rapid growth such as those which have just been considered, but also regions of diminished activity in which the true character of the neoplasm is readily recognized. Here the fibrous stroma is increased in amount and the blood vessels, products of tumor cell activity, are numerous, resembling those found in the benign angiomata. Generally such vessels are capillary in type, although larger cavernous spaces are not infrequently found. Sometimes the endothelial cells in such foci exhibit certain atypical characteristics, such as a primitive, cuboidal form; a crowded arrangement simulating beginning papillary production; and the occasional presence of mitotic figures. They are thus malignant and slowly progressive. In general, however, the cells in the regions of diminished activity are well differentiated and line typical blood vessels. Such sclerosed angiomatous foci represent the oldest parts of the tumor where growth is now quiescent and sclerosis marked.

The large infarcted tumor of the spleen is thought to be of the same nature as the smaller lesions already described. Here for a long period of time there was evidently localized neoplastic growth with the formation of the circumscribed mass. The information

which can be gained from critical observation of sections from the tumor, leads one to believe that it does not differ essentially from the younger and more characteristic lesions already described. It is thus only one of many tumors, differing merely in age and size.

Summary and Conclusions

1. There is here described a case of primary malignant hemangioma of the spleen which metastasized to the liver, with the production of innumerable tumor masses in that organ.

2. The type cell is the endothelial cell and the tumor and its metastases are characterized by the formation of blood vessels and blood-filled spaces. The latter are of atypical and unusual appearance. They exist as small or large, cyst-like vascular cavities into which there project varying numbers of remarkable papillary processes which are covered with rapidly growing endothelial cells. These processes differ from any structures previously described.

3. Neoplastic growth in these foci is active, malignant, and invasive.

4. Alternating with the regions of rapid and atypical growth there are other foci in which typical blood vessel formation is more evident. Here neoplastic activity is diminished or quiescent and marked sclerosis is present. In these areas the growth assumes the form of fibrosed hemangiomata.

5. The tumor is remarkable for its rapid, invasive growth; its unusual histological structure; and the formation of multiple metastases.

6. A review of the literature concerning other cases of malignant, metastasizing hemangioma is given.

I wish to thank Dr. F. B. Mallory for his kind advice and assistance and for making the photomicrographs.

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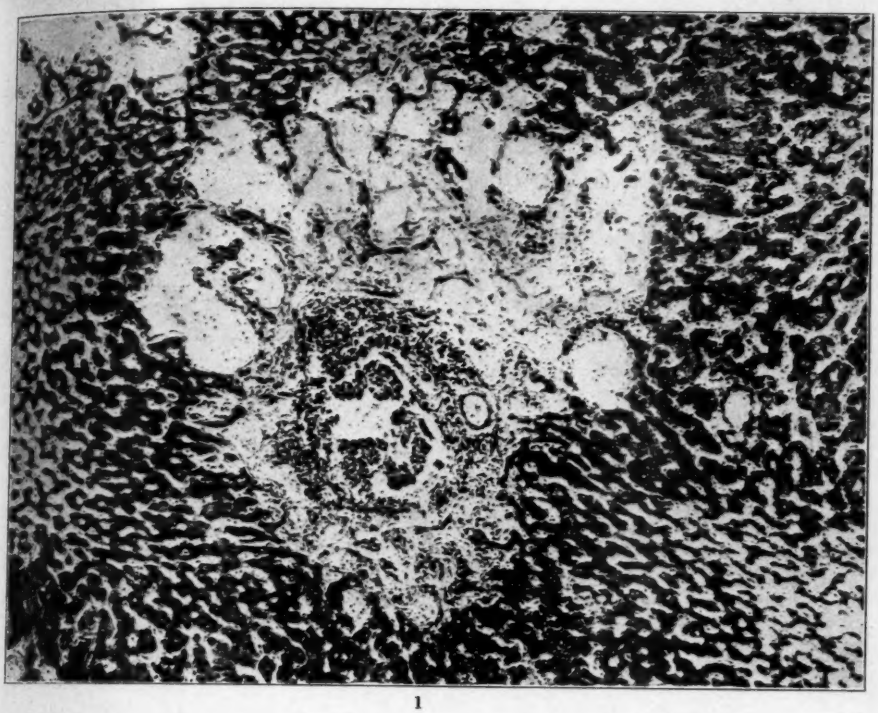
DESCRIPTION OF PLATES

PLATE 115

FIG. 1. Liver. A nodule showing three types of growth, cavernous, capillary, and papillary. Tumor cell proliferation is most active in the last-named region. × 80.

FIG. 2. Liver. Large papillary nodule compressing surrounding parenchyma. Note the complex maze of papillary processes within the dilated space. Blood cells are present in moderate numbers between the projections. × 250.



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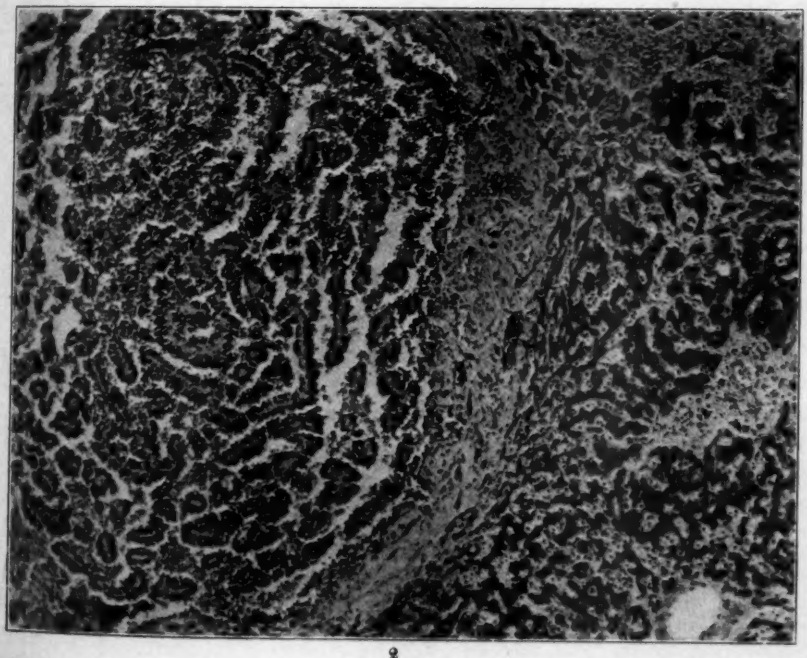


2

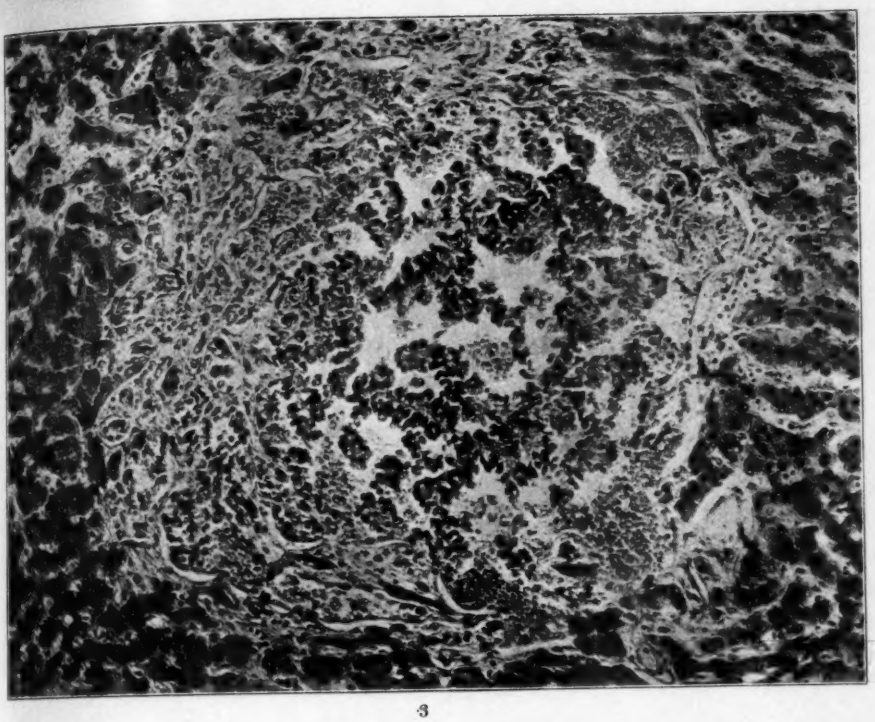
Wright

Primary Multiple Hemangioma of Spleen

Plate 116

Fig. 3. Liver. A nodule in which the peripheral tissue is sclerosed. In the center, however, is a mass of papillary projections on which the tumor cells are growing rapidly. × 150.

Fig. 4. Liver. An early lesion showing how the tumor cells adapt themselves to preëxisting vascular stroma. Sinusoids are dilated and lined with neoplastic endothelium. Liver cells are stretched and atrophic. There is no increase of stroma. × 250.



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4

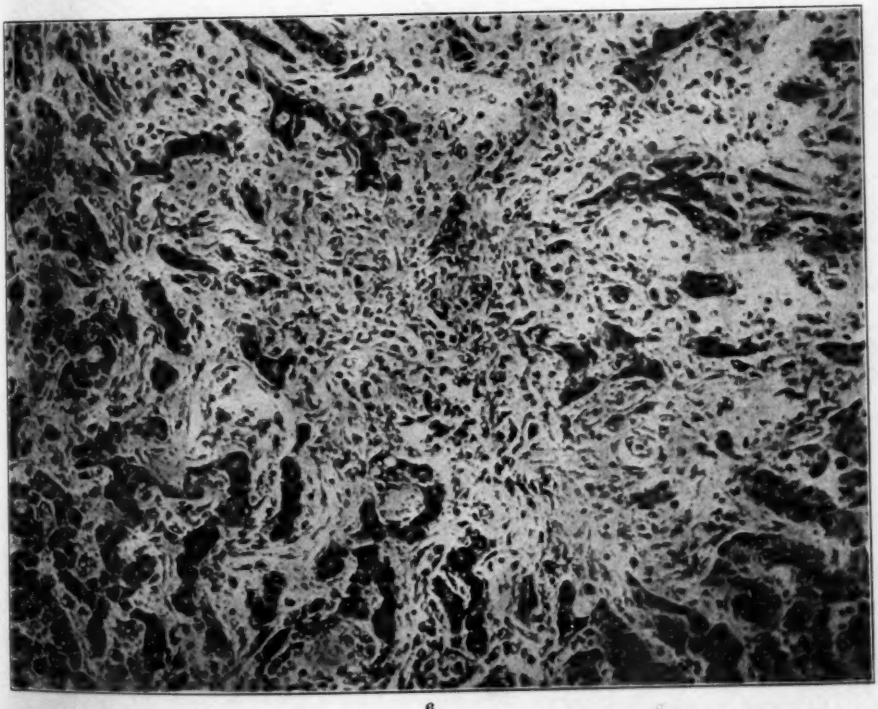
Wright Primary Multiple Hemangioma of Spleen

PLATE 117

FIG. 5. Liver. A relatively late lesion. Many of the liver cells have disappeared. Those which remain are stretched and atrophic. Note the increase in stroma and relatively few vessels. The lesion is becoming scirrhous in character. × 250.

FIG. 6. Liver. A late stage of scirrhous lesion in which many liver cells have disappeared. Note the increase and contraction of the fibrous stroma. The vessels are small and inconspicuous. × 200.

5



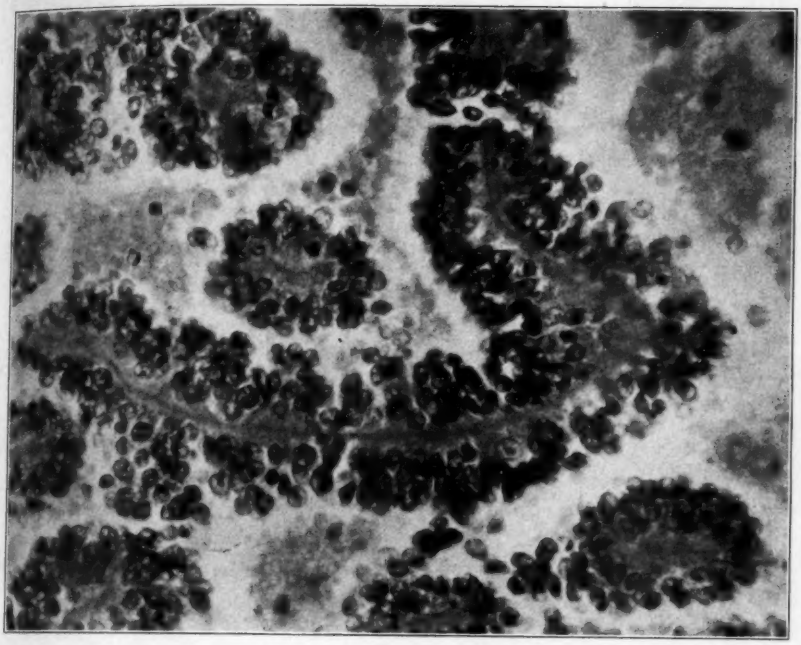
6

Wright Primary Multiple Hemangioma of Spleen

Plate 118

Fig. 7. Liver. A higher magnification of the papillary processes to show their structure. These are non-vascular collagenous strands which are lined with the tumor cells. Note their piled-up, disorderly arrangement and the lack of cytoplasm. × 500.

Fig. 8. Spleen. A high magnification of one of the splenic lesions to show three mitotic figures which indicate rapidity of growth and hence malignancy. In this section the tumor appears as a capillary hemangioma. × 1000.



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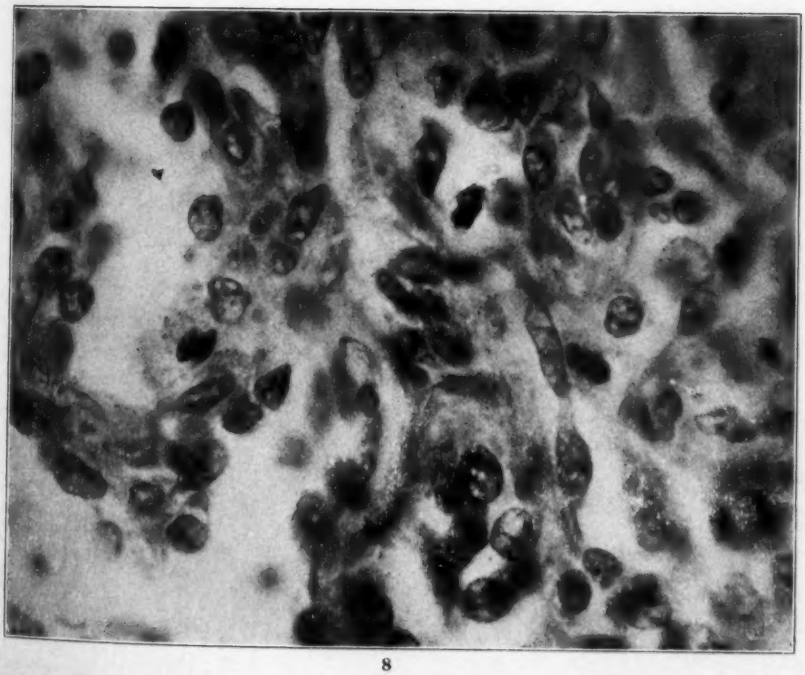

8

Wright

Primary Multiple Hemangioma of Spleen

CHEMICAL CONTRASTS BETWEEN COLLAGENOUS AND RETICULAR CONNECTIVE TISSUE*

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INTRODUCTION

It is now some thirty years since Mall [1] first described reticular tissue and differentiated it from the collagenous by means of tryptic digestion; it is almost twenty since a number of German investigators popularized the Bielschowsky-Maresch technic of impregnating reticulum with silver salts and studied its distribution. In spite of this, very little is known as to the chemical nature of collagen and reticulin. Shortly after Mall published his work, Siegfried [2] made a chemical analysis of the residue of pigs' intestinal submucosa after subjecting it to tryptic digestion and obtained the substance he named "reticulin." Mall had noted that reticular tissue did not yield gelatin on boiling with water and that it was more resistant to tryptic digestion than was collagen; he differentiated the two on the basis of these peculiarities and upon their morphological dissimilarity. Young [3] contradicted Mall's contention, stating that the two substances were chemically identical as they both yielded gels after extraction with hot water. Siegfried, in turn, found that, although a substance resembling gelatin could be obtained by boiling reticulum in water, this was not true gelatin, but something more labile and more readily extracted; furthermore, there was a residue that could be precipitated with ammonium acetate or common salt, a grayish powder, his "reticulin." The gelatinoid material was obtainable after a short period of boiling, formed a firm gel, but differed from gelatin in that it yielded a heavy precipitate with acetic acid, formed no glutamic acid after boiling with hydrochloric acid for seventy-two hours, and contained phosphorus, which is absent in pure collagen and gelatin. Hammarsten [4] gives the following composition for tendon collagen and reticulin, the latter being cited from Siegfried's paper:

* Received for publication June 9, 1928.

Reticulin: C, 52.88; H, 6.97; N, 15.63; S, 1.88; P, 0.34.

Collagen: C, 50.75; H, 6.47; N, 17.87; S, 0.57; P, none.

Siegfried's work was challenged in 1902 by Miss Tebb,[5] working under Halliburton, and her paper elicited an answering article from Siegfried[6] in the same journal and the same year. He had performed additional experiments to reinforce his statements and devised a more rapid method for isolating reticulin, by boiling the tissue in 0.05 per cent hydrochloric acid. His ideas have been accepted by Hammarsten and by Robertson[7] and are quoted in their respective text-books. Abderhalden,[8] on the other hand, contents himself with the mere statement that reticulin is probably an altered form of collagen, less readily convertible into gelatin, thus admitting a chemical difference between the two, but reversing Siegfried's opinion as to the ease with which reticulin is converted into gelatin.

It is difficult to find any definite statements on this subject, even in recently published text-books, although it is probable that much might be found in the voluminous literature on leather and glue research, could one but sift out the more fundamental articles from those of a technical or commercial type. Indeed, the subject seems to elicit very little curiosity in the minds of those who deal with these two connective tissue components.

In the realm of histology and histopathology, the question has been equally slighted; Strong and Elwyn's[9] latest edition of Bailey's text-book and Prentiss and Arey's[10] Text-Book of Embryology merely state that reticulum differs from collagen in its resistance to peptic digestion and in its staining reactions. The histological text-book mentions the possibility of reticulin's being a precurser of collagen. Miller,[11] in his study of the pulmonary reticulum, has said that this is a probability and, in subsequent publications and private conversation, has adhered to this belief. That reticulum is of a somewhat different chemical composition from collagen has been generally assumed by investigators of reticular tissue, but the very close relationship between collagen, reticulin and elastin has always been kept in mind.

Last year, Mallory and Parker[12] seriously questioned any chemical difference between collagen and reticulin; they consider that the silver method is incapable of differentiating two chemically unlike groups of fibers — it merely impregnates those that are readily accessible to the silver bath and fails to penetrate the denser, closely

felted and hence inaccessible bundles of collagen. They conclude: "Reticulum as a chemically distinct intercellular substance does not exist; it is collagen in separated form, rendered prominent by the silver stain." This statement, if generally accepted, closes the door on any further discussion of the subject; it is the purpose of this paper to prevent this by showing that there are chemical differences between reticulin and collagen and that they present a fertile field of investigation to the biochemist. So far as ascertainable, no one has attempted to link the biochemical with the microchemical method of attacking this problem, and it is proposed, in the following report, to do just that. The work will be divided into an histological and a biochemical section and the two will be correlated in the discussion; the spleen has been chosen as the organ of election for this undertaking, other organs being used for confirmatory and secondary objects of investigation.

HISTOLOGICAL SECTION

TECHNICAL METHODS

Methods of Fixation: 10 per cent neutral formalin (4 per cent formaldehyde); 10 per cent formalin with 0.5 per cent HCl and 0.5 per cent NaOH (to test out acid and alkaline fixation); Zenker's fluid; Bouin's fluid; absolute alcohol, acetone and ether (respectively); coagulation with boiling water and alcohol.

Sections: Paraffin embedding was used as a routine, although frozen sections were employed in part of the investigation and celloidin was used in the case of the Bouin-fixed tissue and frequently flooded over sections to prevent their becoming detached from the slides.

Impregnations and Stains: The Hortega silver-ammonium carbonate method was used as a routine impregnation, either alone or with a counterstain of Harris' hematoxylin and Van Gieson's stain.

Directions for Impregnating: Wash the deparaffinized sections in distilled water, after removing mercuric chloride with alcoholic iodin and hypo, in the usual manner. Chromium salts may be removed with $KMnO_4$ and $COOH_2$ (see later). After washing in distilled water, impregnate the sections for 15 minutes in a silver-ammonium carbonate bath which is made up as follows: to 10 cc. 10 per cent $AgNO_3$, add 40 cc. 5 per cent Na_2CO_3; allow the white precipitate to settle, decant the supernatant fluid, wash with distilled water and

redecant, dissolving the precipitate by adding, drop by drop, just enough strong NH_4OH to put it almost into solution, leaving five or six gray granules undissolved; make up to 100 cc. with distilled water. Although the impregnation may be carried out in the cold, it is more satisfactory to warm the bath to the steaming point, driving off the excess NH_3, and cool to 50° C. Then place in an incubator at 37° C, or thereabout, for 15 minutes.

Wash the sections in two or more changes of distilled water and reduce them with 1 per cent formalin, for two minutes. Wash at the tap and tone for two minutes in 1:500 aqueous AuCl, which tends to render the impregnation more precise and converts the yellowish brown collagen into grayish pink, or old rose. After washing, the sections are treated with 5 per cent hypo ($Na_2S_2O_3$) to remove excess silver or gold. The process depends upon the impregnation of the tissue by the double salt of silver and ammonia and its conversion, probably by reduction, into a black compound. That the double salt is silver-ammonium oxide, rather than silver-ammonium carbonate, is probable. It is usually advantageous to counterstain the sections for five minutes in Harris' hematoxylin followed by forty-five seconds in Van Gieson's picric acid-acid fuchsin solution, with a wash between. The sections should be run directly into ascending strengths of alcohol, otherwise water will decolorize the acid fuchsin. Van Gieson's solution is best made up of 10 per cent of 1 per cent aqueous acid fuchsin to 90 per cent of picric acid, water saturated at room temperature.

Staining Characteristics: The simple, untoned impregnation colors reticulum black, collagen yellowish brown; toned with gold chloride, the reticulum is still black, the collagen grayish pink to old rose; counterstained with Van Gieson, the reticulum is unaffected, while the collagen becomes bright vermilion. If the reticulum be unimpregnated with silver salts, it will stain rose-red with Van Gieson's solution.

Other Silver Methods: Another Hortega "variant," in which saturated Li_2CO_3 replaces the Na_2CO_3; the Bielschowsky-Maresch silver-ammonium oxide method, as modified by Mènard and myself;[13] the Achucarro silver tannate method (Foot[14]) and some minor modifications of these were all employed as controls. No marked difference could be detected between these and the routine procedure, while many of them are more laborious and complicated. It may be said,

however, that the modified Bielschowsky-Maresch method gives the most reliable and clean-cut pictures, if one wish to devote the time to it.

HISTOLOGICAL EXPERIMENTS

Effects of Fixation: It was found that, of the fixatives mentioned, only one made any marked difference in the resulting impregnation: absolute alcohol prevented the blackening of the reticulum fibers almost completely; all the other fixatives gave good results and acted very similarly. In order to determine why absolute alcohol should inhibit the impregnation, a set of simple experiments, aimed at lipin extraction, were performed.

Unfixed Frozen Sections: Fresh spleen was cut on the freezing microtome and treated by the routine method; no impregnation of the reticulum or collagen resulted, although the refractile fibers were clearly visible.

Alcohol-Fixed Frozen Sections: Sections of fresh spleen were then soaked in absolute alcohol and ether for two hours, after which they were fixed to slides with celloidin and took the usual impregnation (Fig. 1). Where the sections were thin, the reticulum seemed broken up and the argyrophil material was diffused through the tissue; where they were thick, the impregnation was satisfactory. Next, sections were soaked in this mixture over night and treated as usual; the reticulum no longer became impregnated, there were a few black beads and clumps of reduced silver (Fig. 2). To test the fixation, sections were stained with hematoxylin-Van Gieson, with good success.

Paraffin Sections: Tissue fixed in ether, absolute alcohol, absolute acetone and 5 per cent NaOH was subsequently fixed in 10 per cent neutral formalin and embedded in paraffin. The sections from this material became impregnated very much in the usual way, excepting in the case of spleen treated with NaOH, in which the reticulum failed to become black; the collagen was unaffected. This rules out the possible extraction of lipins that might combine with the silver as a factor in the failure of alcohol-fixed tissue to become impregnated, for all these fluids are fat solvents. Some other explanation must, therefore, be sought.

Fixation in Boiling Water or Alcohol: Boiling water was used as one of the types of fixation to be tested, but it proved more interest-

ing as an extractor. Thin blocks of spleen were boiled for 4.5 hours under a reflux condenser in distilled water; half of them were then removed and run into paraffin, the remaining half being again boiled for the same length of time in absolute alcohol. To avoid injuring the knife on these bone-hard blocks, they were softened over night in 80 per cent alcohol three parts, to glycerol one part. This rendered them soft and they cut more easily than the water-boiled blocks.

The following changes were noted in sections from these two lots of tissue after impregnation: in the water-boiled spleen the reticulum was clumped into swollen, somewhat dendritic masses of black material (Fig. 3), occupying the normal site of that tissue. The collagen was *quite unstained*, a counterstain with Van Gieson's solution brought out a faint roseate tinge in the capsule and trabeculae, but the bright vermilion was no longer forthcoming. The elastic tissue now appeared as isolated, sinuous strands; if the impregnation were preceded by treatment with the oxidizing mixture ($KMnO_4$)+ ($(COOH)_2$) these took on a brilliant orange. Sections from the alcohol-boiled spleen were quite similar to those fixed in cold absolute alcohol.

EFFECT OF OXIDIZING AND REDUCING AGENTS UPON THE IMPREGNATION OF RETICULUM

I have noticed, in experimenting with silver impregnations of brain tissue, that the process was definitely affected by previous treatment with oxidizing and reducing agents. Mènard, in this laboratory, and the technicians at the U. S. Army Medical Museum at Washington (personal communication) have found that reticulum will impregnate better if first treated with permanganate of potash and oxalic acid. As this might depend upon (*a*) oxidation, (*b*) a salt of manganese, (*c*) oxalic acid, both oxidizing and reducing agents; a manganese salt and oxalic acid alone were applied to reticular tissue fixed in a variety of ways (see "*Fixation*") in order to ascertain what differences would result.

(*A*) *Oxidizing Agents:* $KMnO_4$, 0.25 per cent, for five minutes, followed by oxalic acid [$(COOH)_2$] for ten, was first tested. The reticular impregnation was sharply intensified; fibers not demonstrable after simple formalin fixation (Fig. 4) became black and clear-cut after "oxidizing" (Fig. 5). These photomicrographs were from serial sections from the same block of tissue. The treated section

showed fibers that were somewhat stouter than those in the controls, the collagen was pinkish, if not counterstained with fuchsin, somewhat lighter than the controls if this were used; the cytoplasm and nuclei became paler and insignificant, which is an advantage — for they may then be better counterstained with organic dyes. The same feature was demonstrable with Zenker-fixed material; the untreated controls showed somewhat sharper impregnation of the reticulum than did the formalin-fixed controls, and the cytoplasm was brownish as a result of the formation of silver chromate; the "oxidized" sections showed sharper impregnation of the reticulum and markedly inhibited impregnation of the cytoplasm and nuclei. It is this fact that makes our (Foot,[12] Foot and Mènard [13]) modifications of the Hortega method peculiarly adapted for use with routine, Zenker-fixed material. In Bouin-fixed spleen, the results were materially the same, but after alcohol fixation, the impregnation of the reticulum was always slight, although the counterstain took very well. Subsequent fixation of alcohol-fixed tissue in 10 per cent formalin rendered the tissue suitable for first-rate silver impregnation. The reaction involved in the permanganate-oxalic acid treatment does not indicate oxidation on the face of it, as will be seen from the following:

$2\ KMnO_4 + 8(COOH)_2 = 2\ Mn(COO)_2 + K_2(COO)_2 + 10\ CO_2 + 8\ H_2O$.

Other Oxidizing Agents: Sections were treated respectively with commercial peroxide of hydrogen, acidified $KClO_3$ (1 per cent with 0.5 per cent HCl) and 30 per cent $NaNO_2$ with HCl to acidity. They were then impregnated as usual, always with untreated sections as controls in each lot. The reticulum was no better, if as well impregnated in the oxidized sections, although the nuclei were more sharply and precisely demonstrated. This would seemingly point away from oxidation as a factor in the improvement seen after the $KMnO_4 - (COOH)_2$ treatment, but it might depend upon the more feeble oxidizing power of these reagents.

(*B*) *Potassium Permanganate Alone:* This was tried alone in 0.25 per cent concentration for five minutes. After impregnation, as compared with untreated controls, the manganated sections were much better, but rather spotty and filled with a brownish deposit from the manganese salts; the reticulum was, on the whole, more sharply impregnated. As the silver-bath contains NH_4OH, this

would oxidize the $KMnO_4$ as follows: 2 $KMnO_4$ + 2 NH_4OH_4 = 2K · NH_4MnO_4 + H_2O + Oxygen; continuing: 2 K · NH_4MnO_4 + 2 H_2O = 2 MnO_2 + 2 KOH + 2 NH_4OH + 2 Oxygens. This, then, may be construed as a true oxidation.

Potassium Permanganate and Hydrochloric Acid: In order to test out a more powerful oxidizing combination, $KMnO_4$ was used as usual and followed by ten minutes in from 1 per cent to 3 per cent HCl. The reaction here is: 2 $KMnO_4$ + 6 HCl = 2 $MnCl_2$ + 3 H_2O + 5 Oxygens. The permanganate was not bleached out of the sections by the HCl as it was by the $(COOH)_2$, and the resulting impregnations were very dense. The reticulum was probably even better blackened than in the oxalic acid technic, but the background was so intensely impregnated that the contrast was very slight and the reticulum obscured. Compare Figs. 6 and 7, made from the same block, the former with oxalic, the latter with hydrochloric, acid.

Inert Manganese Salt: It might be thought, in spite of this, that the superior results after $KMnO_4$ were due to the presence of the Mn ion, therefore sections were treated with $KMnO_4$ + HCl, as above, for a control and with $MnCl_2$, followed by HCl. As compared with the controls, the $MnCl_2$ sections were vastly inferior, which would rule out the Mn ion as a controlling factor.

(*C*) *Oxalic Acid Alone:* As this is a constituent of the most successful method, it was used alone for 10 minutes in 5 per cent concentration. There was no impregnation of the reticulum, although it was well demonstrated in the controls. This should be compared with the results obtained with the reducing mixture, as given later on, for oxalic acid is a feeble reducer.

EFFECTS OF REDUCING AGENTS ON IMPREGNATION

A mixture of pyrogallol and NH_4Br, 0.5 per cent each and thiosulphate and sulphite of soda, 1 per cent each (in distilled water), was applied to sections for 15 minutes in warm solution before impregnating them with silver; this totally inhibited any impregnation of the reticulum (Fig. 8), except when absolute alcohol had been the fixative, when a partial impregnation (superior to untreated controls) resulted. The mixture of reducing agents was found to be superior to any one of them used separately. The function of the NH_4Br is not known; it possibly restrains the reduction, as in photography. While this is not striking proof, it at least strengthens the

assumption that the oxidation effected by $KMnO_4$ is the explanation of the improved impregnation following its use. (*Cf.* "*Oxalic Acid Alone.*") Alcohol-fixed tissue probably impregnates better after treatment with the reducing mixture for the same reason that it does after treatment with 10 per cent formalin, which is a reducer. The function of reduction and oxidation in this process must be further investigated; it is very bewildering.

EFFECT OF THE HYDROGEN ION CONCENTRATION OF THE FIXATIVE ON IMPREGNATION

As acids figure largely in the pretreatment of sections when $KMnO_4$ is used, it might be considered as the determining factor. Against this assumption is the unsuccessful impregnation after $NaNO_2$ and $MnCl_2$, both of them followed by HCl. To strengthen this proof, sections were made from spleen fixed in 10 per cent formalin to which was added 0.5 per cent HCl in the one case, 0.5 per cent NaOH in the other. In both instances the sections became fairly well impregnated, but treatment with $KMnO_4$ and $(COOH)_2$ produced the usual brilliant and superior impregnation; acidity or alkalinity of the fixative, therefore, appear to play no part in the process.

OTHER SILVER IMPREGNATIONS

The action of $KMnO_4$ and acid was found to improve the impregnation of sections by the Bielschowsky-Maresch technic, the Hortega Li_2CO_3 variant and the Achucarro silver tannate technic; the reducing mixture inhibited impregnation in all these methods.

REMARKS

All this indicates that it is best to treat sections with $KMnO_4$ before impregnating with silver. This should be followed by 5 per cent oxalic acid, if a light background suitable for counterstaining be desired; it should be followed by 1 per cent to 3 per cent HCl, if a denser impregnation is wanted and counterstaining is dispensed with. The great difficulty, in this method, is the liability of the sections becoming detached from the slides in the silver bath. This may be avoided (*a*) by careful drying of the mounted sections in the paraffin oven for forty-eight hours and a day or two at room temperature; (*b*) by driving off the excess NH_3 in the silver bath be-

fore impregnating, cooling to 50° C before the sections are immersed; (*c*) by removing the slides from the "descending absolute alcohol," after deparaffinizing in xylol, and flooding them with very thin celloidin (as in Mallory and Wright's frozen section technic), allowing this to set and continuing with the hydration, through 95 per cent alcohol.

BIOCHEMICAL SECTION

Preliminary Experiments: The first thing to do was to check up on Siegfried's original findings, therefore, splenic tissue was treated as he treated intestinal submucosa. Human spleen was used for two reasons — it contains more reticulum than most organs, has plenty of collagen for comparison and had been used in the experiments just recorded in the histological section. Spleens, fresh from the necropsy table, were ground in a meat mill, thoroughly washed in water, followed by several changes of distilled water and digested for two or three days with 0.25 per cent Parke, Davis & Co. pancreatin and 0.5 per cent $NaHCO_3$, in distilled water at 37° C. The solution was filtered off each day through a kitchen strainer, renewed, and toluol added to keep down putrefaction. Finally the tissue was washed in 0.05 per cent HCl and boiled in the same concentration of the acid under a reflux condenser for two or three hours. The connective tissue broke up into a muddy precipitate, mostly without going into complete solution, although there was considerable reprecipitation on adding NaCl.

The precipitate and broken-down tissue were dried by gentle heat and some of the material was fixed in formalin, run into paraffin and sectioned; when stained, the sections showed some unmistakable tissue-débris, bits of capsule, trabeculae, consolidated reticulum and the like (Fig. 8). The remainder was extracted with chloroform and alcohol, but the extracts were so crude that more exact methods were used later. Thus far the results bore out Siegfried's statements in many particulars; there was far more tissue débris than he described and the watery extract would not jell. Siegfried did not examine his reticulin with the silver method, which had not yet come into being, so it was imperative to use it here. The bits of capsule and trabeculae were found to contain argyrophil fibrils and there were clumps of black material adhering to their margins, evidently compacted reticulum. This stained blue with Nile blue

sulfate, showing that it was not blood, which is faintly greenish yellow with that dye. Extracted "reticulin" showed somewhat less argyrophil material when impregnated with silver salts. There was a good deal of elastic tissue in the residue, which was to be expected, as it is very resistant to tryptic digestion and weak acids.

Following this experiment, a dozen or more were run off, each one representing increased refinement in technic and giving somewhat different results. Over ten kilograms of spleen were thus treated. It was found best to slice the organ, rather than to grind it, cutting slices about 2 mm. thick. It was also found necessary to wash the material repeatedly after digestion, in distilled water, in order to get rid of the last traces of digested cellular material. This was best accomplished by rotating the splenic framework in a liter of water in a beaker, using a glass stirring-rod belted to a low speed motor.

All the vicissitudes of these experiments need not be recorded here, but it would be well to indicate that a bewildering number of substances were isolated in earlier trials, subsequent improvement in technic showing many of them to be impurities. Siegfried's "reticulin" was recovered by boiling the digested splenic framework in dilute HCl, as already noted, the "collagen" fraction being separated by tedious filtration. It was found, however, that the following method, in which the "reticulin" was completely dissolved in NaOH and reprecipitated by neutralization and salting out, gave the best yield of the two fractions and simplified their separation.

ROUTINE METHOD FOR ISOLATING RETICULIN

About half a kgm. of the organ, at a time, was sliced, washed and digested in the incubator with either Parke, Davis & Co's., or Squibbs' pancreatin, 0.25 per cent to the liter of distilled water and 0.5 per cent sodium bicarbonate. After twenty-four hours' digestion at 37° C, the foul fluid was run off through a strainer, the material that was held back was washed in the mechanical separator for an hour or two in frequent changes of water, followed by distilled water, and then put back to digest in fresh fluid for another day. This usually sufficed; if not, another day's digestion was enough. The splenic framework, now reduced to an oyster-colored network of lacy reticulum and coarser trabeculae, was given a final

cleaning in several changes of tap water, followed by distilled water, in the washing machine, and it was then boiled in 200 cc. of distilled water under a reflux condenser for two or three hours. The tissue shrank considerably and gave off a yellow extract, *the aqueous extract.*

Following this, the extract was filtered off and set aside, the residue being rinsed in distilled water and digested in the incubator with 0.5 per cent NaOH for twelve or more hours. A peculiar greenish brown, fluorescent fluid, with scraps of undissolved connective tissue (elastin?) as a sediment, resulted. The residue was filtered out and again treated with NaOH, to insure as complete solution as possible, the dissolved material being pooled with that of the first run.

If the reader recall the histological section, the reason for these two steps will be clear; it was found that hot water extracted all the fuchsinophil material from the splenic trabeculae and capsule, while NaOH extracted the argyrophil material from the reticulum. What more natural, then, than to boil first in water to extract the "collagen" and follow this by digestion with NaOH to extract the "reticulin"?

The alkaline solution was then filtered through two thicknesses of filter paper in a Seitz vacuum filter, refiltered through four layers and then through an asbestos disc; the filtrate was deep brown, the process time-consuming. Hydrochloric acid was then added carefully to the filtrate until it was just acid to litmus paper. A putty colored precipitate immediately formed and was much increased by adding NaCl in considerable amounts, 5 per cent. The precipitate was then removed by centrifugation and dried over a water-bath, whereupon it became an amorphous, blackish powder. This was extracted for four hours in a Soxhlet apparatus, with 95 per cent alcohol, which removed a heavy extract of deep brown lipins. This was followed by extraction with distilled water for a like period, to remove the salt and any water-soluble material that might remain. Capryllic alcohol was added in small quantity, to keep down the troublesome foaming. The extracted residue was then dried over a water-bath, becoming a dark brown, somewhat crystalline powder.

DESCRIPTION OF FRACTIONS

Aqueous Extract: When evaporated to dryness over a water-bath, this yellow, water-soluble fraction became a glue-like sheet of brittle, hygroscopic material that powdered into flat, shining, brown flakes. On dissolving this in a little water, spreading on a microscopic slide, drying and fixing in Zenker's fluid, it could be handled like a section. After the routine silver impregnation and Van Gieson counterstain, it became bright red and showed no argyrophilic material; there were fibrillar artefacts, resulting from coagulation, that resembled collagen fibrils. With Van Gieson's stain alone, it stained rose-red; with acid fuchsin, somewhat deeper; with Mallory's connective tissue stain it became light blue; with Weigert's elastic tissue stain, pinkish; with silver carbonate alone, yellow-brown to amber. Commercial gelatin, treated in the same way, stained yellow with picric acid in Van Gieson's solution, orange with the orange G. in Mallory's stain, and deep brown with silver carbonate alone. Therefore our aqueous extract apparently differs from gelatin.

It gave a heavy, flocculent precipitate with tannic acid and a lighter one with Zenker's fluid; gold chloride, hydrochloroplatinic acid and other heavy metals produced varying degrees of turbidity. It did not reduce Fehling's solution and gave a pinkish biuret reaction. Its reactions, therefore, resembled those of pure gelatin to a certain extent.

Sodium Hydroxide-Soluble Fraction: Under the microscope, before alcoholic extraction, this appeared as a mixture of amorphous yellow granules, brownish plates and small crystals. Under the polarizing microscope, the crystals were anisotropic. Some of the yellowish granules proved to be iron-containing pigment, derived from splenic deposits and readily removed by treatment with HCl. The anisotropic crystals disappeared after alcoholic extraction. The partially purified precipitate, after neutralizing the alkaline solution, when fixed and impregnated on a slide, became impregnated with silver and did not take the acid fuchsin of the counterstain. It was composed of two sorts of material, black granules or even threads, and yellowish brown amorphous masses with some crystalline material, presumably salt (Fig. 9). The fibrils were sometimes continuous, sometimes beaded,and the impregnated and counterstained smear showed a striking resemblance, in its color scheme, to sections

impregnated by the Hortega method. Unimpregnated control smears showed no black material and the threads were invisible until impregnated with silver. These were not always obtained, sometimes the argyrophil matter was purely granular, particularly in the purified reticulin.

Alcohol Soluble Fraction: This extract was a deep mahogany-brown. On cooling, a precipitate settled out, identified as a mixture of glycolipins. It was very inconsiderable in bulk, reduced Fehling's solution and gave a modified Pettenkofer's test with sulphuric acid, without the addition of sugar. Dried on a water-bath, about one-third of the remaining material was found to be acetone soluble and to contain fats, fatty acids and some cholesterol; it gave a positive Salkowski reaction. The remaining two-thirds, soluble in cold alcohol and insoluble in acetone, was found to be composed of impure lecithin; it gave a Pettenkofer reaction with sugar and sulphuric acid, became whitish and swelled in water, and formed myelin figures when properly treated. These lipins gave very unsatisfactory impregnations with silver as they were difficult to hold on glass slides and the pictures were rather atypical. They do not seem to be the determining factor in the argyrophilia of the reticulum.

Remarks

It may be said, then, that it is possible to extract a material with the staining properties of the collagen of histological parlance, by boiling the digested framework of the spleen in distilled water; it is also possible to dissolve the residue from this in weak caustic soda and to reprecipitate a substance from this solution by neutralizing with hydrochloric acid and salting-out with sodium chloride. Alcohol extraction removes lipins from this, leaving an insoluble residue which may be redissolved in sodium hydroxide, reprecipitated by acidifying and purified of the contained salts and water-soluble material by extraction with distilled water in a Soxhlet extractor. The purified product is very similar to Siegfried's description of his "reticulin."

This method was applied to spleen as a routine measure, but similar treatment of lung and heart, both rich in reticulum, gave precisely similar results, excepting that there was a copious, insoluble residue (resisting boiling in weak acids or alkalis) in the case of the lung, presumably largely composed of elastin.

In short, extraction with hot water has been found to remove the fuchsinophil material from connective tissue fibers and NaOH to remove the argyrophil. Hot water extract, on evaporation and fixation in Zenker's fluid, is strongly fuchsinophil; sodium hydroxid extract, when dried and purified of the lipins and water-soluble substances, is argyrophilic (after formalin fixation and treatment with $KMnO_4$ and $(COOH)_2$) and does not stain with fuchsin. This would argue that there are two extractable substances in connective-tissue, leaving elastin out of the reckoning; the one may be extracted without destroying the fibers, the other (recovered after alkaline digestion) probably representing the digested fibers and the argyrophil material that coats, or is closely bound up with them.

Discussion

What are "Reticulin" and "Collagen"? These terms must first be defined, a difficult task.

Reticulin: (*a*) From Mall's standpoint: a tissue that resists tryptic and peptic digestion and gives no gelatin on boiling with water.

(*b*) From Siegfried's: a tissue composed of two substances that resist tryptic digestion; one of them soluble in boiling, weak hydrochloric acid and gelling on cooling, the other precipitating out with ammonium acetate or sodium chlorid. Reticulin contains phosphorus.

(*c*) From the point of view of the histologist: any reticular connective tissue becoming black when impregnated with double salts of ammonium and silver.

(*d*) According to Mallory and Parker: merely a separated, more compactly fibrillary form of collagen fiber.

(*e*) From the viewpoint of many of us, frankly agnostic: it is a possible forerunner of collagen, very closely related to it, but chemically different; it might be oxidized, or hydrolyzed collagen, or collagen plus or minus some side chain, or an isomer of collagen.

There is little need for discussing these further, with the exception of the definition of Mallory and Parker, which is too categorical and is, apparently, backed by very little chemical proof. If the substances were chemically identical and their differences in staining merely a matter of physical accessibility to the silver-bath, we should expect: (*a*) that the surface of the section, all of it equally accessible to the fluid, would have a superficial, mirror-like, black

coating, which it does not; (*b*) that the two substances would be extracted by the same solvents, again untrue; (*c*) that the action of oxidizing and reducing solutions would affect both in equal degree, which is contrary to the facts.

It should be understood that reticulin and collagen are apparently very closely related from a chemical standpoint and that the differences in their staining reactions, solubility, etc., depend upon presumably trifling differences in their chemical composition — the one seems to glide over into the other in the most gradual sort of transition. For this reason, the statements made in the paper of Mallory and Parker are probably not much overstressed, but their explanation of these phenomena on a purely physical, or mechanical basis seems to be unwarranted.

The origin of these fibers need not detain us here, for we have not been investigating that aspect of the subject; suffice it to say that the precipitation of argyrophil fibrils from a solution that was filtered several times, argues against any but a chemical, or secretory agency on the part of fibroblasts, or other cells of mesenchymal type.

Collagen: Definitions of collagen may be briefly discussed and dismissed.

(*a*) From the standpoint of the chemist: a substance with a somewhat variable chemical formula and hydrolyzed into gelatin on boiling with water over long periods. It contains no phosphorus.

(*b*) From the standpoint of the histologist: a tissue component that stains red with acid fuchsin and blue with anilin blue (disregarding its other staining characteristics) and is found in the white fibrous connective tissue.

To elaborate further: the chemist believes that the collagen fiber is gradually broken up and converted into a jelly (gelatin) by hydrolysis, so that his conception of collagen includes the fiber; the histologist believes that any fibrous tissue with the characteristic staining reactions mentioned is collagenous, hence he implies that the whole fiber is composed of collagen, just as the chemist does.

But we have seen that there is, apparently, a more complicated arrangement of the components of collagenous tissue; the fuchsinophilia disappears after boiling the tissue in water without the fibers being destroyed, the collagen fiber apparently gives up a substance with which it has been impregnated, or saturated, without itself going into solution.

Conclusions

We may, then, speculate on the probable significance of the data set forth in this paper. These would indicate that the connective tissue is composed of a protoplasmic ground substance which is stained by ordinary acidophil dyes such as eosin, picric acid, etc. After extraction with boiling water and weak caustic soda, there remains a stainable framework. After brief extraction with alkaline solution, the collagenous trabeculae and capsule, and the reticulum of the spleen are still fuchsinophil, but the reticulum is no longer argyrophil; after extraction with boiling water alone, this fuchsinophilia disappears, but the argyrophilia remains. Hence it appears that the fibrous and fibrillar framework is composed of one substance and that all this is associated with a water-soluble, fuchsinophilic material; that the reticular tissue contains in addition to this an alkali-soluble argyrophilic substance.

On leaching connective tissue, freed of cellular material by tryptic digestion, in weak NaOH over a considerable period, the fibrous ground substance is also broken up to a greater or lesser extent. Therefore our "reticulin," recovered by chemical isolation, would be a mixture of the protoplasmic ground substance of the fibers *plus* the very scanty argyrophil matter that characterizes reticulum. This is borne out by the silver impregnation, which shows a brownish yellow background of amorphous material in which black granules and (or) threads are demonstrable. Furthermore, in one batch of tissue that was forgotten and left in the NaOH solution in the incubator for four days, the "reticulin" that was recovered showed considerable fuchsinophilia in smears, which indicates that the reticulin had been hydrolyzed by the NaOH into collagen, an assumption quite in accord with the idea that reticulin is a forerunner of collagen. Young tissue shows more of the former, old tissue more of the latter; take cicatrization as an example.

Phosphorus tests on the isolated "collagen" and "reticulin" fractions were very unsatisfactory; the collagen gave a heavy precipitate with ammonium molybdate in acid solution; this dissolved in ammonia, giving a greenish fluid, and reappeared as a bluish green precipitate on adding nitric acid. The reticulin was quite insoluble in acid, unless boiled in concentrated nitric acid; it gave a questionable precipitate with the molybdate, and the addition of ammonia

changed the fluid to a deep orange-brown. This must be worked out more carefully by a skilled chemist. As Siegfried used different methods and as we have seen that our reticulin is apparently a mixture, while our "collagen" is admittedly different from the usual conception of collagen, the presence or absence of phosphorus is relatively unimportant.

There is another point that is not in accord with the usual conception of collagen and gelatin and this is the almost complete failure on my part to obtain any jellies in the aqueous fractions. This might be explained on the basis that the tryptic digestion had removed most of the gelatinous material from the connective tissue; the combination of digestion with very thorough centrifugal washing may be responsible. Once in a while partial jelling of the aqueous fractions would occur, but not often. A review of the literature will show the greatest lack of unanimity on the part of Mall, Young, Siegfried and Tebb on this very question.

In closing, it should be noted that this work is merely a beginning and that there remains a great deal to be done, as the reader has probably already discovered; it is hoped that the results here set forth may interest some skilled biochemist, who will be in a position to prosecute the chemical investigation and identification of the substances isolated by the methods herein described far more successfully than could a pathologist, who lacks the requisite intensive training in biochemistry and the critical chemical judgment that are essential in running down and identifying the various substances involved.

The kindly and ready assistance of various members of the Department of Biochemistry of our College of Medicine has helped the writer over many hard places in this investigation and is most gratefully acknowledged. Messrs. Mènard and Homan have been very coöperative in aiding with the technical details of impregnation and the photomicrography.

Summary

1. In the connective tissue framework of the human spleen, isolated by means of tryptic digestion, there are three main groups of fibrous substances: (*a*) "collagen," (*b*) elastin and (*c*) reticulin.

(*a*) The first may be completely extracted by boiling water, leaving the white fibrous tissue incapable of taking specific collagen

stains. The extract may be coagulated on slides by means of gentle heat and fixation in Zenker's fluid and a substance will be recovered that gives all the characteristic staining reactions of collagen and many of the precipitation tests of gelatin.

(*b*) The elastin resists boiling water, and weak acid or alkaline solution. It may be digested with pepsin and HCl, 0.3 per cent. (This was not mentioned in the body of the paper; it is given for the sake of completeness.)

(*c*) The reticulin is composed of a mixture of at least three groups of substances; an alcohol-soluble group (lipins, largely impure lecithin), an alcohol-insoluble group made up of an argyrophil material that may come down as threads and a silver neutral background of amorphous matter that forms the bulk of the fraction; this may be digested fibers.

2. After extracting these substances from the splenic framework, if alkali-digestion be not carried too far, pale fibers that stain neither with silver nor fuchsin remain; these may be stained with eosin, picric acid, or phosphotungstic acid hematoxylin; rose, yellow, and reddish respectively. ("True collagen?")

3. The staining reactions of the substance extracted with boiling water and those of commercial gelatin are not the same.

4. Using the technic herein described, jellies are very seldom produced.

5. The argyrophil matter in the reticulin is best demonstrated after exposure to certain oxidizing agents, while reducing agents apparently inhibit impregnation.

6. There is a possibility that the water-soluble "collagen" and the alkali-soluble "reticulin" constitute stiffening, strengthening, or protective substances for the fibers of the connective tissue; they impregnate these evenly and may be extracted without destroying the fibers.

7. The assumption that reticulin may be hydrolyzed to collagen is not unwarranted.

NOTE: All but the last two figures represent photomicrographs taken with a 16 mm. objective and 10x ocular.

Photomicrographs by author and Prof. J. B. Homan, of the Department of Medical Art, College of Medicine, University of Cincinnati.

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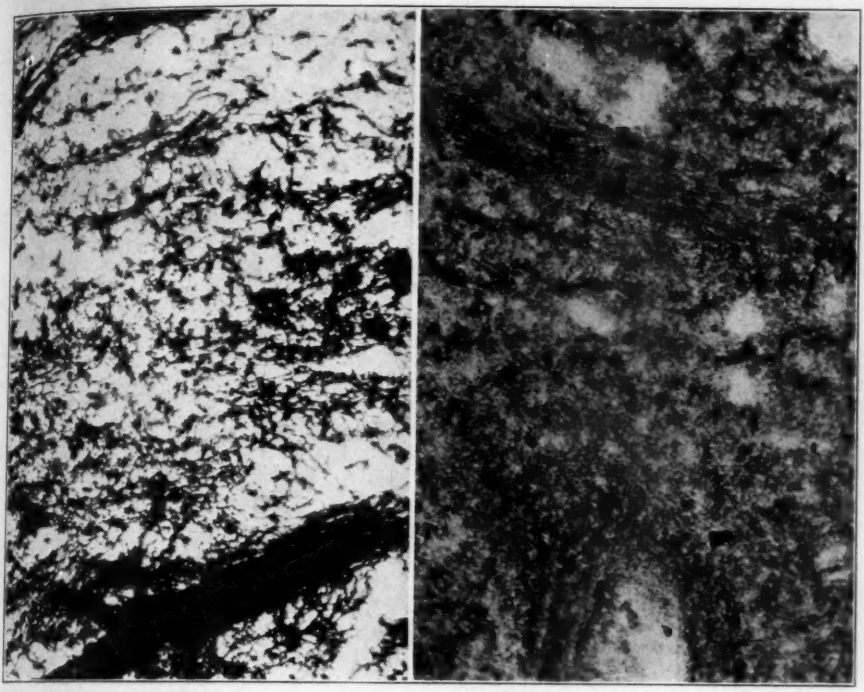
DESCRIPTION OF PLATES

Plate 119

Fig. 1. A frozen section of fresh spleen, subsequently fixed for two hours in absolute alcohol and ether, *aa*. Simple silver impregnation. Reticulum fairly well impregnated.

Fig. 2. Another frozen section from the same lot, after soaking 20 hours in absolute alcohol and ether. Silver impregnation. Note that the reticulum is no longer well impregnated, although the section is from the same block as the preceding. A Van Gieson hematoxylin stain on a control section from this batch, after 20 hours in alcohol and ether, took perfectly.

Fig. 3. A paraffin section of water-boiled spleen, silver-Van Gieson technic. The unstained trabeculae should be noted; when they no longer take acid fuchsin they photograph white, instead of black, and the elastic tissue and reticular fibers in them become evident. Notice the masses of coagulated reticulum, well impregnated, in the splenic pulp.



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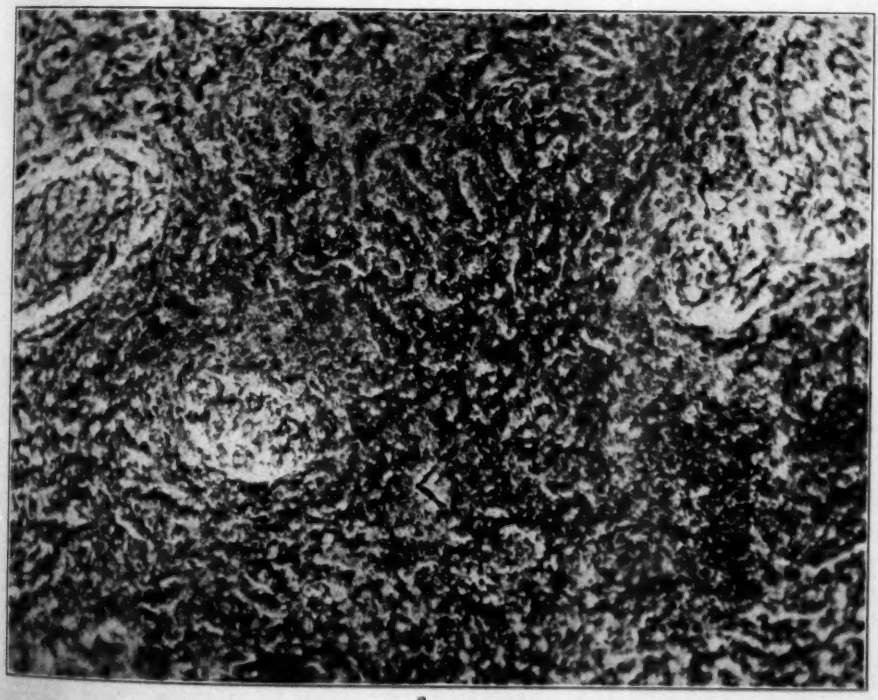

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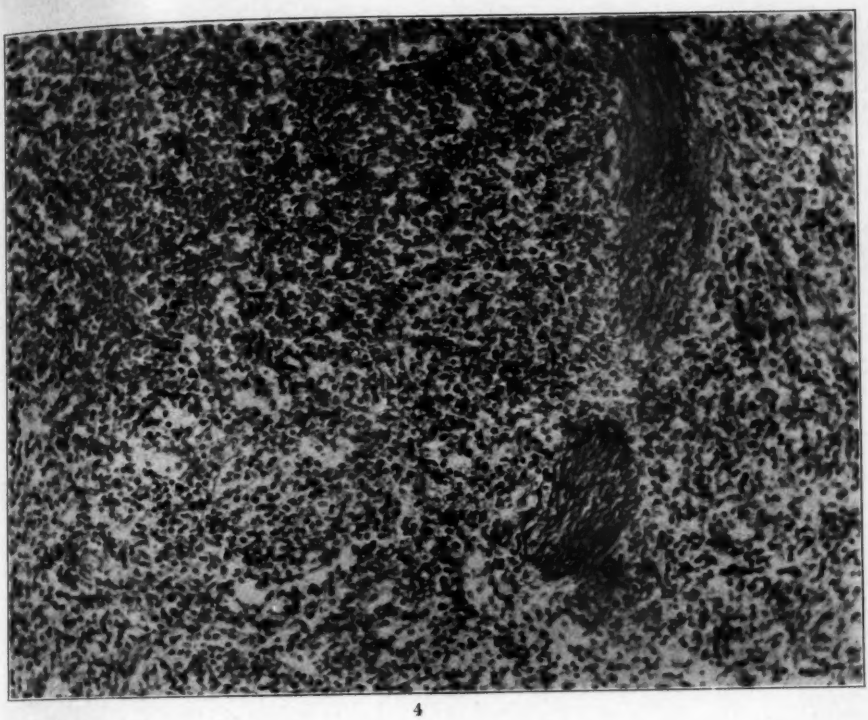
Foot

Collagenous and Reticular Connective Tissue

PLATE 120

FIG. 4. A section of formalin-fixed spleen after silver-Van Gieson impregnation. No pre-treatment with permanganate of potash and oxalic acid. This is an unusually poor impregnation.

FIG. 5. Serial section from same block as Fig. 4 after silver impregnation *preceded* by permanganate of potash and oxalic acid. The difference in the impregnation is self-evident.



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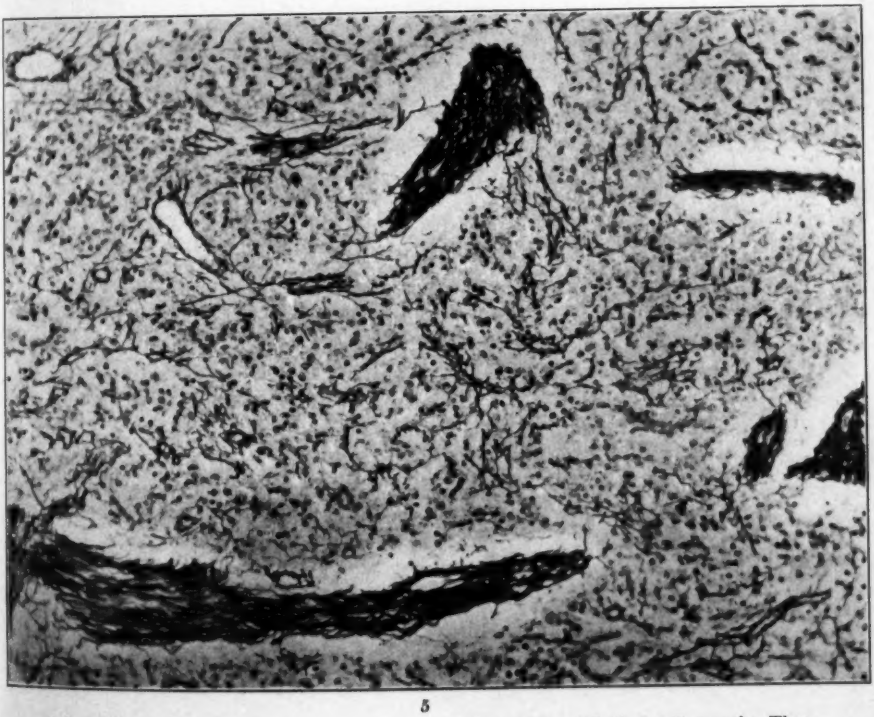

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Foot

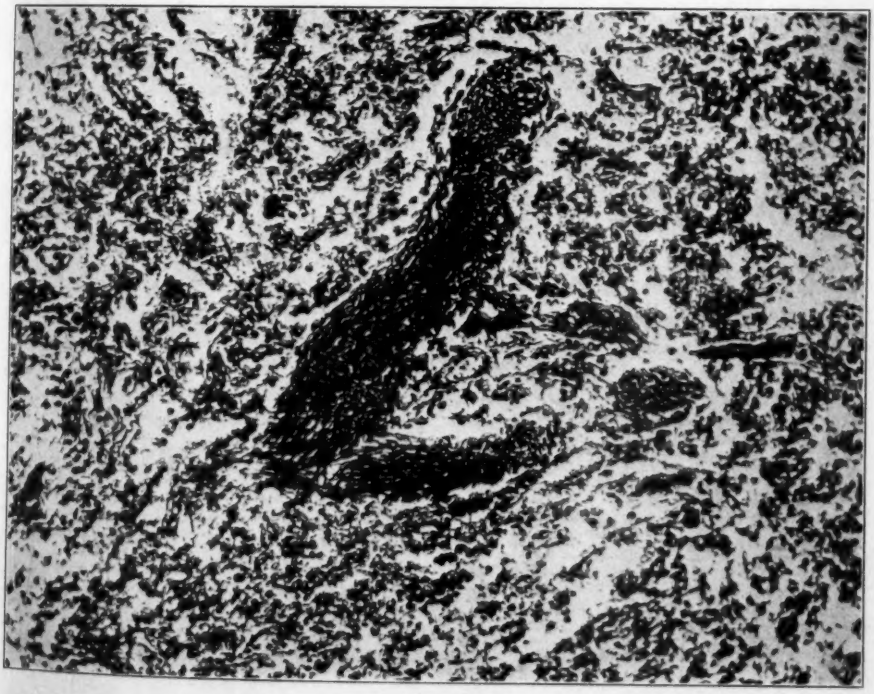
Collagenous and Reticular Connective Tissue

FIG. 6. Same technic as preceding, but from another block of tissue; for comparison with Fig. 7.

FIG. 7. From same block as Fig. 6, with the oxalic acid replaced by 3 per cent hydrochloric acid. Note the totally different type of impregnation resulting from the continued oxidation.

(Figs. 4 to 7, inclusive, were from sections in which the Van Gieson counterstain was omitted.)

6



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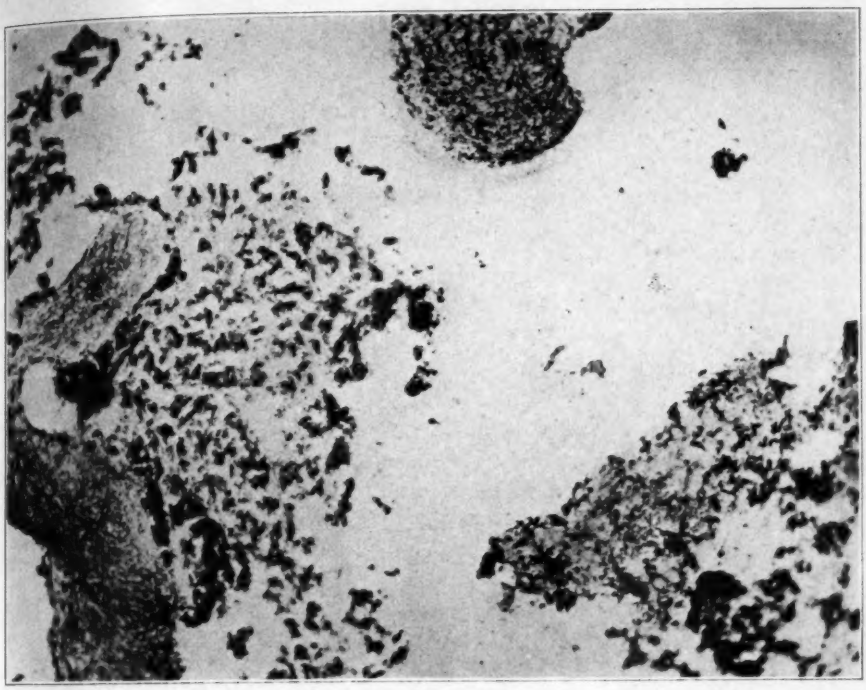
Foot

Collagenous and Reticular Connective Tissue

PLATE 122

FIG. 8. Paraffin section of a mass of precipitate resulting from the boiling of isolated splenic framework with weak HCl. The tissue-like character of the material is perfectly evident. Silver-Van Gieson technic.

FIG. 9. Silver-Van Gieson impregnation of a smear of the precipitate obtained by neutralizing and salting-out the alkaline solution of isolated splenic framework after filtration through filter-paper. The fibers are presumably thrown down by the reprecipitation. Note the disparity in their size and configuration, the beaded appearance of some of them and their general resemblance to reticulum fibers as shown in the preceding figures. The yellowish brown background has been largely eliminated by the yellow-green filter used in taking the photomicrograph.



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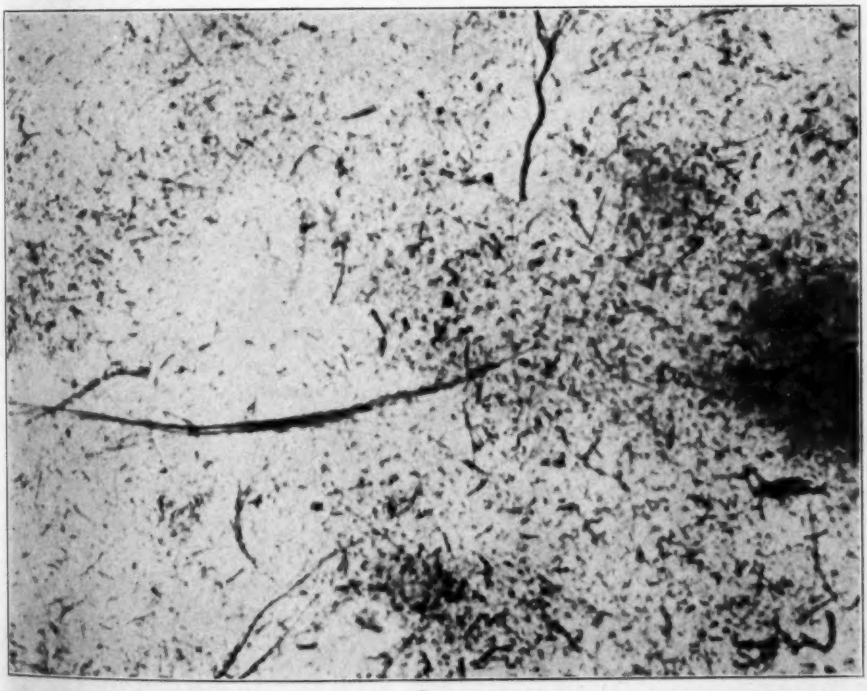

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Collagenous and Reticular Connective Tissue

STUDIES IN ACROMEGALY *

VII. THE MICROSCOPICAL STRUCTURE OF THE ADENOMAS IN ACROMEGALIC DYSPITUITARISM (FUGITIVE ACROMEGALY)

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INTRODUCTION

It is the purpose of this paper further to describe a pituitary disorder that appears to be associated with an acidophilic adenoma of foetal type and in which the constitutional evidences of glandular oversecretion are so slight or so masked by signs of glandular insufficiency that they have usually been disregarded. For this disorder we have come to employ the designation *fugitive acromegaly*.

More than twenty years elapsed after Marie [18] gave its name to acromegaly before it was possible to say with any assurance that the hypertrophic features of the malady he had described were actually pituitary in origin and in all probability an expression of glandular overaction. Another twenty years passed before this hypothesis received definite proof through the production first of experimental gigantism in rats (Evans and Long [13]), and more recently of acromegalic gigantism in the dog (Putnam, Teel and Benedict [19]) by the parenteral injection of extracts of the anterior lobe.

In the interval the chief arguments in favor of this hypothesis were based almost wholly on the negative evidence of experimental canine hypophysectomies (1908–9) which showed that adult animals (Crowe, Cushing and Homans [5]) became thereby adipose and sexually dystrophic and that young animals (Aschner [1]) remained dwarfed and sexually infantile. As these disturbances were obviously due to deprivation of glandular secretion, it was natural to assume that the overgrowth of acromegaly and gigantism represented the opposite state due to an excess of secretion. This assumption received further support from the apparent subsidence in size in the extremities of the first few acromegalic patients that had been successfully operated upon (Hochenegg,[14] Cushing [9]).

* Received for publication August 10, 1928.

Though there was still considerable confusion in regard to the independent function of anterior as opposed to posterior lobe and though the hypothalamic syndrome was as yet unappreciated, on the basis of these experimental and clinical observations the senior author in 1909 [6] ventured to describe pituitary disorders as divisible in a general way into states of hyper- and of hypopituitarism. The existence of intermediary or transitional states was however recognized and in the following year an address on the subject [8] was concluded with the statement:

"It may be expected: (1) that in all cases of original hyperpituitarism associated with tumor, the functional end-result will be hypopituitarism; and (2) that in many of the cases in which existing hypopituitarism is the striking feature traces at least of an early tendency to hyperpituitarism can be detected. These mixtures of the two syndromes of glandular over- and under-activity are most conveniently designated as *dyspituitarism*."

In a subsequent monograph [7] dealing with pituitary disorders in general, chief attention was paid to the mechanical effects of intra- and extrasellar tumors of various sorts on the structures in the neighborhood of the gland and but little attention to the histopathological nature of the lesions themselves. As a matter of fact, at that time (1912) the character of the glandular enlargement even in acromegaly was but poorly understood and frequent recourse was had to the non-committal term *struma* in describing it. These "strumas," particularly when associated with hypopituitarism, were recognized as having adenomatous characteristics and to be composed either of chromophobe or of chromophile cells, but in the case of acromegaly it was not clear whether the lesion represented a diffuse functional hyperplasia of the chromophilic elements of the anterior lobe or whether it was actually an adenomatous tumor composed of these elements.

Even Benda,[4] who in 1901 was the first carefully to study and compare the hypophysial changes in a series of acromegalics, is somewhat obscure in his description of the lesion which he called *struma hyperplastica* or *adenomatosa*; for though he recognized in three of his four cases "the extraordinary increase of granular (acidophilic) gland-cells" in the lesions, he says that:

"The acromegalic hypophysial tumors diverge in unmistakable ways from the habits of other diffuse organ-hyperplasias. On the one hand the tumor-character is outspoken even in the smaller tumors by the sharply cut boundary of

the growth; on the other hand histologically the arrangement of the growing cells is neither absolutely like that of the adult nor of the foetal organ. It remains an atypical form which sometimes reaches the border of what we must call malignant."

Unfortunately subsequent investigators were unable to substantiate Benda's views; whether this was from failure to employ sufficiently specific methods of treating the tissues or because the hypophysial "strumas" they chanced to study were not from cases of actual acromegaly is not now possible to determine. The latter is the more probable explanation since more than ten years elapsed before it came slowly to be recognized that by far the most common form of pituitary tumor was unassociated with acromegaly and was a chromophobe type of adenoma which had been regarded previously as a sarcomatous process.

In 1921 Bailey and Davidoff,[3] in the study of the tissues which had been secured at operation in this clinic from thirty-five patients with full-blown acromegaly, found, in complete agreement with Benda, that almost without exception they were adenomas whose cells contained acidophilic granules, though in some cases these so-called alpha-granules were present only in small numbers in scattered cells. Four years later, in another paper from this clinic dealing from a general standpoint with pituitary adenomas of all sorts, Dott and Bailey[12] came to the conclusion that not only might an acromegalic show signs of pituitary insufficiency late in the course of the malady but that "in many cases at least, the features of acromegaly and of the hypopituitary syndrome develop synchronously rather than in sequence." They singled out from the series which then comprised 162 verified adenomas, thirteen examples of what, in want of a better term, they called "mixed types," the clinical history of one of the patients having been included (Case VII) in their report.

Material and Methods

In the hope of shedding further light on this obscure "mixed" syndrome, other examples of which have since come under observation, we have undertaken the present study with the purpose of determining whether it might be possible to correlate the degree of eosinophilia found in an adenoma with the coëxistent clinical picture. With this end in view the tissues from a hundred consecutive

adenomas that had been operated on between December 21, 1921, and January 1, 1927, and from which sufficient material for study had been secured and properly fixed, were re-examined. Almost without exception the tumors had attained sufficient size to affect vision by pressure on the chiasm, which was the primary reason for the operation. We are therefore not here concerned with those rare instances of acromegaly, of which Lewis [17] and more recently Krumbhaar [16] have recorded examples, due apparently to a functional hyperplasia of the anterior lobe without actual tumefaction.

The method for identification of the alpha-granules already described by one of us,[2] of using on Regaud-fixed tissue the neutral dye made by a combination of ethyl-violet and orange-G, was employed throughout.

Classification

From a purely histological standpoint and with complete disregard of the associated clinical symptoms, such a series of adenomas may be divided into six groups with the typical granular chromophilic adenoma of acromegaly at one end of the scale, the typical non-granular chromophobe adenoma at the other end, and with four intermediary types of gradations, as follows:

Type I (Fig. 1). This is a highly eosinophilic adenoma which consists of large, polygonal or rounded cells that lie in a loose mass in which there is no stroma of connective tissue and practically no vascular sinuses. The cells which have distinct boundaries vary greatly in size and possess an abundant coarsely granular cytoplasm, often transformed centrally into a hyaline mass so that many nuclei are excentrically situated and crescentic in form. The nuclei are vesicular and show great variability in size. Multinucleated cells are numerous. Crescent cells are also found. *Practically all the cells are packed with alpha-granules, often of large size.*

Type II (Fig. 2). This also is a definitely eosinophilic adenoma though of less exaggerated type. The cells have the same sharp boundaries, abundant cytoplasm and vesicular nuclei of varying size. There is no connective tissue stroma and vascular sinuses are rare. The general structure however tends to be more compact so that the cells are largely polygonal and the cytoplasm has rarely undergone the central hyaline change with resultant excentric position and deformation of the nuclei. Multinucleated cells are also less fre-

quently encountered. *Although many non-granular cells are present most of them contain large numbers of alpha-granules.*

TYPE III (Fig. 3). The tumors of this group are those described by Dott and Bailey [12] as "mixed adenomas." Like the above they consist of a compact mass of polygonal cells with well defined boundaries and vesicular nuclei. The cells however have less abundant cytoplasm and multinuclear cells are rarely found. There is no stroma but vascular sinuses are rather numerous. *In the periphery of a variable number of the cells a thin ring of alpha-granules is demonstrable.*

TYPE IV (Fig. 4). The tumors of this group differ from the preceeding in that the nuclei are not so consistently vesicular and *no alpha-granules are demonstrable.* Without a specific stain for the granules it would scarcely be possible to distinguish these adenomas from those of Type III.*

TYPE V (Fig. 5). In these tumors there may sometimes be a poorly developed stroma, but the vascular sinuses are fairly numerous and the elongated neoplastic cells sometimes appear to radiate from them. Cellular boundaries are rather indistinct. The relatively scanty cytoplasm of the cells is less deeply stained than in the cells of Type IV and the nuclei are of uniformly smaller size and have a heavier chromatinic network. *No alpha-granules are present.*

TYPE VI (Fig. 6). These adenomas are characterized by a stroma of connective tissue carrying numerous small thin-walled vascular sinuses, dividing the growth into broad cellular columns. The neoplastic cells are elongated or spindle-shaped and often stand at right angles to the septa of connective tissue. They have indistinct boundaries, a feeble staining cytoplasm, and oval, small nuclei containing a heavy chromatinic network. *No alpha-granules are present.*

ATYPICAL CASES. A chromophobe adenoma which is composed of such narrow columns of cells separated by numerous vascular

* Attention should be drawn to the fact that there is some evidence of differentiation in certain of the non-granular cells especially in some of the tumors of this Type IV. If a fragment of such a tumor be immersed in a 1 per cent solution of methyl green and examined under the microscope, the nuclei of the cells will usually be stained bright green and the cytoplasm light yellowish green. However, a variable number of cells, depending on the tumor, will be stained a bright violet and their nuclei remain colorless. The nuclei of these violet cells seem larger than the others. In fixed preparations stained by ethyl-violet, the periphery of these cells retains the dye with considerable tenacity although no definite granules are found. These facts seem to indicate that the chromophobe cells are not all of the same nature.

sinuses that it resembles the normal structure of the anterior lobe is occasionally met with. In those cases of acromegaly also in which there is only a slight enlargement of the hypophysis a somewhat similar structure may be found. These exceptionally rare adenomas have been listed as "atypical."

In the order in which the hypophysial adenomas have been thus subdivided or graded, the cells become smaller, the boundaries less distinct, multinucleated cells disappear, the nuclei become more pycnotic, the cytoplasm less well stained and the stroma and sinuses become more abundant. But more important than all else is the fact that in the descending scale the highly granular cells of Type I have passed to a non-granular type.

Clinical Correlation

When the hundred adenomas were subdivided on the basis of the foregoing histological differentia without reference to the associated clinical syndrome, the following result was obtained, the most striking feature of the tabulation being the numerical preponderance of the purely chromophobe types.*

Type I	8 cases
" II	8 "
" III	11 "
" IV	11 "
" V	47 "
" VI	12 "
Atypical	3 "
Total	100

After this histological study had been completed, the voluminous clinical histories were then independently abstracted with special attention to any recorded signs or symptoms that might possibly be interpreted as evidence of a fugitive hyperpituitary effect. It was found that:

1. The sixteen adenomas of Types I and II proved in all in-

* This preponderance of chromophobe cases in the series can in part be accounted for by the fact that the chromophobe adenomas are more likely to impair vision than the adenomas of acromegaly and consequently are for this reason more likely to be operated upon. Though the thick walled sella of acromegaly is capable of distention, comparatively speaking the chromophilic adenomas are less favourable for operative intervention by the usual transphenoidal operation than are the chromophobe adenomas.

stances to have been accompanied by definite clinical acromegaly and in general the more numerous the alpha-cells, the more pronounced was the acromegalic syndrome. To this rule, however, there were the following exceptions: In six of the cases with outspoken acromegaly numerous alpha-cells had been demonstrated in the tissues, but they seemed to be fewer than the intensity of the clinical picture would have led one to expect. There were, however, in these cases some extenuating circumstances. In two of them the tissues were fixed in Zenker's fluid and it is possible that more alpha-cells were present than could be demonstrated. In two other cases there was evidence that the acromegalic process was inactive at the time of the operation.

2. The twenty-two transitional adenomas of Types III and IV with which we are chiefly concerned proved to be associated in many instances with clinical indications of an abortive hyperpituitary action. This was almost invariably true of the eleven Type III adenomas which showed occasional cells with a peripherally disposed thin ring of alpha-granules. In this group enlargement of hands and feet was recorded in six instances; coarsening of the features in eight, hypertrichosis in two, normal or exaggerated libido in two, excessive height in three, excessive perspiration in two, squaring and tufting of the phalanges in four, persistent lactation in one, glycosuria in one, and four of the cases had a normal or slightly elevated basal metabolic rate whereas practically without exception the rate in all patients with adenomas of Types IV, V and VI was subnormal, often markedly so.

Among the eleven patients with Type IV adenomas, although no alpha-granules were demonstrable, two prove to have been very tall and heavily built, one had spacing of the teeth, one hypertrichosis and exaggerated libido and another definite tufting of the terminal phalanges.

3. The fifty-nine patients with purely chromophobe adenomas of Types V and VI had given no clinical evidence of anything suggesting even a trace of fugitive hyperpituitarism with three possible exceptions: In two patients with Type V lesions, a suggestive tufting of the terminal phalanges had been mentioned in the X-ray report; and one patient with a Type VI lesion was a tall man with prominent lower jaw but no malocclusion of the teeth. Whether these trifles can be looked upon as suggesting a taint of acromegaly is doubtful.

As a result of this clinico-pathological study it may be stated with reasonable conservatism: (1) that highly acidophilic adenomas are invariably accompanied by unmistakable acromegaly; (2) that the pure chromophobe types are with equal definiteness unassociated with any clinical traces of this disorder; and (3) that an intermediary group of adenomas with sparsely disposed cells containing a peripheral zone of alpha-granules, are likely to produce clinical evidences of a fugitive hyperpituitary reaction.

The histological characteristics of the adenomas of this intermediary group are in other words, sufficiently distinctive to enable the pathologist to state, without knowledge of the clinical symptoms, that the patient probably shows traces of hyperpituitarism. What is more, it is possible for the clinician to predict with some assurance when this intermediary type of adenoma will be disclosed by the microscope. Such a clinical prediction has received histological verification in four cases operated upon during the past year.

Case Reports

It will suffice to give a few briefly abstracted case histories in illustration of the condition that we have come to designate a transitional, abortive or fugitive acromegaly.

Case I

Transitional Adenoma of Type III with Marked Local Symptoms and Dyspituitarism Chiefly Shown by Adiposity in a Man of Large Frame

On *September 18, 1923*, Harry P. (Surg. No. 19695), aged 24, single, a foreman, was referred to the hospital by Dr. L. Solis Cohen of Philadelphia with the diagnosis of pituitary tumor.

History: Prior to 1918, he had always been well. In that year he began to be troubled by indistinct vision, supposedly astigmatic. Shortly afterward he began to gain rapidly in weight from 170 to 220 pounds. During the past six months vision has failed to such an extent that he is no longer able to read print. His secondary sex characters appeared at a normal age; he has always been somewhat sparsely bearded; libido has been undiminished during the present illness. There has been no polyuria or polydipsia, no headache, no loss of mental vigor.

Examination: This disclosed a bilateral primary optic atrophy, a complete bitemporal hemianopsia, and a greatly enlarged and deepened sella turcica. The basal metabolic rate was −5 per cent. No glycosuria, no polyuria.

The patient was a heavy-boned, rather obese young man (Figs. 7 and 8), 5 ft. 10 in. tall and weighing 225 pounds. His skin was smooth and hair was scanty over face and chest. His extremities were disproportionately large. He wore a 7⅞ hat, a 11½ shoe and No. 9 glove. The features were coarse and the

lips thick; there was maxillary rather than mandibular prognathism. The cranial X-ray showed the accessory nasal sinuses to be considerably enlarged. The external genitalia were normally developed.

Clinical Impression: Hypophysial adenoma with dyspituitarism.

Operation: On *September 27, 1923,* the transphenoidal approach to the distended sella proved to be difficult because of ossification of the cartilage of the septum, and the presence of hypertrophic spurs such as occur in acromegaly. The exposure of the distended sellar floor was consequently difficult. About a thimbleful of soft adenomatous tissue was removed.

Course of Illness: There was no immediate postoperative improvement in the visual fields but the acuity increased from 20/200 to 20/50 and he was discharged on *October 4, 1923.* He was given a series of X-ray treatments with further improvement in vision which has been retained for five years. When last heard from on *June 29, 1928,* he reported himself to be in excellent health and weighing 20 pounds less than in 1923, so that his malady may be considered stationary.

Microscopical Examination: Fixatives: Regaud, Zenker. Stains: M.V.E., P.T.A.H., E.V.O.G. The tumor is composed of polygonal and elongated cells with abundant granular cytoplasm (Fig. 9*a*). The nuclei are large and vesicular. An occasional multinucleated cell is seen. Vascular sinuses are rare and there is no tendency to form a stroma of connective tissue. With E.V.O.G. stain a few cells are seen with blue granules in the periphery of the cytoplasm (Fig. 9*b*).

Pathological Diagnosis: Transitional adenoma of Type III.

Comment: Although this patient might have passed in 1923 as having a mild hypopituitary syndrome, the fact of his unusually large extremities with a heavy skeleton aroused the suspicion that there might be an acromegalic taint and this was further supported by the hypertrophic condition of the nasal septa encountered at the operation. The pathological diagnosis at the time of the operation was that of pituitary adenoma (unqualified) and its foetal eosinophilic type was not recognized until the tissues were more recently reviewed.

Case II

Large Transitional Adenoma of Type III with Dyspituitarism in an Adipose Woman of Large Frame

On *November 3, 1924,* Ida H. (Surg. No. 22521), a farmer's wife, 37 years of age, was referred to the hospital by Dr. William R. Goff of Parkersburg, West Virginia, with the diagnosis of pituitary tumor.

History: The patient had been a vigorous woman though susceptible to recurring attacks of "quinzy" until her tonsils were removed in 1921. After bearing three healthy children her menses ceased. This occurred in 1914, ten years before entrance, and was ascribed to a preceding attack of typhoid fever, after which she began to grow stout (135 pounds in 1914 to 193 pounds on admission). In 1922 she began having bitemporal headaches with progressive obscuration of vision. She has suffered of late from indigestion, dyspnoea, sensitivity to cold and fatiguability with weakness. She has noticed an increasing coarseness of her features and increase in size of her hands and feet.

Examination: This showed a large, obese woman of middle age with heavy features (Figs. 10 and 11) and large hands and feet. The skin was coarse and damp with perspiration. There was pallor of the optic nerves, bitemporal field defects, and a greatly enlarged and deepened sella turcica. The X-ray films of the hands showed "suggestive squaring of the metacarpals but no other evidence of acromegalic change." There was no glycosuria nor polyuria. The basal metabolic rate was +5 per cent. The temperature tended to be subnormal.

The *clinical impression* was that of a hypopituitary state with traces of antecedent acromegaly.

Operation: On *November 14, 1924,* a transphenoidal operation was performed with intrasellar removal of considerable soft adenomatous tissue which was under tension and comparatively avascular. She made a good recovery with both subjective and objective improvement in vision. She was given subsequent X-ray treatments and was discharged on *December 4, 1924.* No subsequent reports.

Microscopical Examination: Fixatives: Zenker, Regaud. Stains: M.V.E., P.T.A.H., E.V.O.G. The tumor is composed of polygonal cells with rather abundant, coarsely granular cytoplasm (Fig. 12*a*). The nuclei tend to be vesicular and often excentrically situated. Multinucleated cells are occasionally seen. Small vascular sinuses are present but there is no tendency to form a stroma. With E.V.O.G. stain a few scattered cells are seen which contain fine blue granules in the periphery of their cytoplasm (Fig. 12*b*).

Pathological Diagnosis: A transitional adenoma of Type III.

Comment: It would appear from the clinical record that the patient was suspected of having dyspituitarism associated with a transitional or fugitive acromegaly though these descriptive terms had not yet come into use. The pathological diagnosis of the lesion, based on routine examination, was again "pituitary adenoma" (unqualified) and it was not until our recent reclassification on the basis of secretory granules that the suspicion of a transitory hyperpituitarism received histological support.

Case III

Dispituitarism with Transitional Acromegaly in a Man with a Large Adenoma of Type III that had Extruded into the Sphenoidal Cells

On *December 17, 1924,* Harold P. (Surg. No. 22822), aged 44, a steamfitter, was referred to the hospital by Dr. William F. Regan of Boston with the complaint of headaches and numbness of extremities.

History: The patient was married and the father of one child 16 years of age. Previously of vigorous health, he for ten years had been having recurring headaches, sometimes so severe they caused vomiting and profuse sweating, obliging him to lay off from work. After a period of remission for a few years, these headaches have returned though in a milder form. During the past three or four years he has gained 50 pounds in weight and there has been a noticeable diminution of energy and ambition together with loss of libido. Meanwhile his vision has been noticeably failing. He has been on an antiluetic treatment.

Examination: He was a powerfully built, ponderous and obese man (Figs. 13 and 14) over six feet in height and weighing 211 pounds. The skin was soft and smooth with scanty axillary and pubic hair and sparse, slow growing beard so that he shaved only once a week. The genitalia were small. The hands and feet were big and broad and though the fingers tapered normally the X-ray disclosed slight tufting of the terminal phalanges. There was no prognathism but the frontal and sphenoidal sinuses were large. The sella was huge, with pressure destruction of the posterior clinoid processes. There was a bilateral choked disc of low grade and left homonymous field defects. The temperature tended to be subnormal; the basal metabolic rate was −11 per cent. No glycosuria or polyuria.

Clinical Impression: Hypophysial adenoma with hypopituitarism and traces of antecedent acromegaly.

Operation: On *January 5, 1925*, a transphenoidal operation was performed in the course of which an hypertrophic maxillary spur such as characterizes acromegaly was encountered. An abundance of soft adenomatous tissue which had already extruded through the sella and was filling the sphenoidal cells was radically removed. The recovery from the operation was uneventful but the headaches continued and the left homonymous hemianopsia progressed. He was given a course of X-ray treatments and was discharged on *January 29, 1925*.

Course of Illness: He reported on *May 29, 1925*, when his visual fields were found to have filled out almost to normal, with a slight residual left upper quadrantal defect. His general condition was much improved and he had gone back to work. On *February 16, 1926*, he was found to have again become apathetic and the defect in his visual fields was larger. Another series of X-ray treatments was given. When last heard from, *July 3, 1928*, it was reported that the patient had had two or three attacks which seemed like convulsive seizures. Otherwise his condition was largely unchanged and he was able to continue with his work.

Microscopical Examination: Fixatives: formalin, Regaud, Zenker. Stains: M.V.E., P.T.A.H., E.V.O.G. The tumor is composed of polygonal and irregular cells with abundant, coarsely granular cytoplasm and sharply defined boundaries (Fig. 15*a*). The nuclei are large and vesicular. A few multinucleated cells are seen. There is no stroma, but vascular sinuses are numerous and in some areas there is a large amount of collagenic tissue, probably secondary to degenerative change in the tumor. With E.V.O.G. numerous cells have a ring of fine blue granules in the periphery of the cytoplasm (Fig. 15*b*).

Pathological Diagnosis: Transitional adenoma of Type III.

Comment: This man obviously had a sufficiently large intracranial extension of his adenoma to cause an increase in intracranial tension probably due to mild hydrocephalus with resultant papilloedema. The tumor had also broken through the floor of the distended sella so that the sphenoidal cells were filled with the growth. Examples of similarly extensive adenomas in cases, however, of unmistakable acromegaly have been recorded by Cushing and Davidoff in a previous paper in this series.[10] In this particular case the mixture of clinical hypo- and hyperpituitary symptoms was sufficiently definite to arouse the suspicion that an adenoma of transitional type

would be disclosed. This prediction was upheld by the histological studies of the tissues.

Case IV

Large Transitional Adenoma with Marked Neighborhood Symptoms and Dyspituitarism with Fugitive Hyperpituitarism Shown by Glycosuria

On *Sept. 30, 1925*, Robert P. (Surg. No. 25120), a civil engineer, 33 years of age, entered the hospital on the advice of Dr. Robert B. Giles of Dallas, Texas, with a diagnosis of pituitary tumor which had been long treated by antiluetic measures and by radiotherapy. The sole complaint was of failing vision.

History: Shortly after the patient's discharge from the Army in *1919*, he noticed a periodical, bitemporal clouding of vision which by *December* of *1923* had markedly increased. In 1924 he began to have fronto-temporal headaches and to gain in weight, which increased from his normal of 125 pounds to 183 pounds by January of 1925.

In 1923, owing to a supposedly positive Wassermann, he was given a vigorous antiluetic treatment; and in July of 1925, by which time his vision had become markedly impaired, he was given X-ray treatment in spite of which vision continued to fail.

Examination: He was a well developed, over-well nourished man 176.3 cm. in height (Figs. 16 and 17). There was considerable puffiness of the face and coarseness of the skin associated with a moderate hypertrichosis. The genital development and libido were normal. Though married he had no children. There was no evidence of skeletal overgrowth either of skull, hands or feet shown by the X-ray. The basal metabolic rate was −18 per cent.

The neighborhood symptoms were pronounced: the sella greatly enlarged; bilateral primary optic atrophy; a temporal hemianopsia in the left eye with 20/100 vision and vision in the right eye reduced to shadows. The patient brought with him a perimetric chart taken three months previously at which time there was a fairly typical bitemporal field defect.

The case would have been looked upon as an example of probable chromophobe adenoma with hypopituitarism had it not been that he was found to have glycosuria, the urine showing 1.42 per cent of sugar.

He had come across the country by automobile and arrived with a most severe case of hemorrhoids which more urgently demanded attention than his hypophysial tumor in spite of his approaching blindness. The hemorrhoids were operated upon on *October 5, 1925*, and from this procedure he made a speedy recovery. He was re-admitted to the hospital a month later.

Operation: On *November 13, 1925*, the usual transphenoidal operation was performed in the course of which it was found that the tumor had extruded into the sphenoidal cells. In consequence of this a much less satisfactory and thorough extirpation of the growth than usual was made and it was not certain how much of the intrasellar portion of the growth had actually been removed. He made an excellent postoperative recovery but unfortunately without the usual marked improvement in vision. However, he subsequently did well and on *July 14, 1927*, he reported that he had not missed a day at his office and was enjoying good health.

Microscopical Examination: Fixatives: Regaud, Zenker. Stains: M.V.E., P.T.A.H., E.V.O.G. Tissue removed consists of a portion of flattened anterior

lobe and small fragments of adenoma. The latter is composed of polygonal cells with abundant, coarsely granular cytoplasm (Fig. 18*a*). Blood vessels are frequent and there is a tendency to form a stroma. The nuclei are vesicular and a few multinucleated cells are seen. E.V.O.G. stain shows numerous cells which contain blue granules (Fig. 18*b*).

Pathological Diagnosis: Transitional adenoma of Type III.

Comment: In spite of his glycosuria, this patient was looked upon as a typical example of mild hypopituitarism and the adenoma had been recorded as chromophobe in type until it was found during our recent re-examination of the tissues that many of the cells had peripherally disposed of granules.

In a previous number of this series of studies,[10] it was stated by Davidoff and Cushing that whereas one out of every four patients with acromegaly shows glycosuria at one time or another in the course of the disease, patients with pituitary insufficiency on the contrary almost invariably show a high carbohydrate tolerance. The presence of glycosuria in this patient should consequently have sufficed to make us hesitate to accept the original clinical and pathological diagnoses that were made. The subsequent disclosure in this case of an adenoma whose cells contain alpha-granules is consequently of particular interest and may possibly afford an explanation of other cases that have been reported in which glycosuria has been observed in patients with supposed hypopituitarism.

Other Cases

It would merely prolong without strengthening this communication should we submit to the temptation of giving the clinical histories of the remaining seven patients in whom a Type III adenoma was found. In all of them the diagnosis of chromophobe adenoma had originally been made and yet, the notes on most of the histories make it apparent that the clinical observers were puzzled by the presence of symptoms that are uncommon in pure hypopituitary states. The usual syndrome was that shown by the first three of the four patients whose histories have been given, namely, suggestive signs of a tendency to overgrowth; but acromegaly in Marie's sense, though the most striking feature of adult hyperpituitarism is by no means its only manifestation. Three of the patients were women who were victimized by an offensive degree of hypertrichosis in association with an exaggerated libido. Another woman following

second pregnancy failed to regain her menstrual function and has continued to lactate for a period of six years; she shows coarsening of the features, is troubled by excessive hyperhidrosis and has gained thirty pounds in weight. The majority of these patients had a basal metabolic rate estimated at slightly above zero per cent, whereas a rate of −20 to −30 is usual in uncomplicated hypopituitarism associated with chromophobe adenomas.

The anterior lobe may possess a dual function with independent principles influencing growth on the one hand and the organs of sex on the other. Time will shed light on this. Meanwhile it is not at all unlikely that failure in the past to recognize fugitive expressions of hyperpituitarism, that are unaccompanied by any clinically demonstrable evidences of skeletal overgrowth, may have been a source of confusion. The cases described in the literature (*e.g.*, Kraus,[15] Case 23) in which an eosinophilic adenoma has been unaccompanied by acromegaly may come to be thus explained.

Discussion

We are aware that the histological studies of the tissues on which our type-grouping has been based are open to criticism on two scores: (1) in that only a small part of the tumor has been subjected to examination and the assumption must be made that the portion removed is representative of the entire growth; and (2) in that the tumors vary in size so that there is no means from the operative specimen of estimating the total number of alpha-cells in a given lesion. In spite of these possible sources of error the correlation between the clinical syndrome and the microscopical structure of the adenoma seems to us to be sufficiently consistent to justify the conclusion that *there is a definite relationship between the number of alpha-granules in a hypophysial adenoma and the intensity of the associated acromegalic syndrome.*

In an attempt, from the standpoint of functional pathology, to interpret the clinico-pathological significance of this association of transitional adenomas with fugitive acromegaly, we may begin with the one fact concerning overgrowth that now has an experimental basis: namely, that the prolonged administration of extracts of the anterior lobe of the hypophysis can produce states in animals comparable to gigantism and acromegaly in man. Almost certainly,

therefore, these latter conditions are due to hypersecretion rather than to a possible perversion of secretion of the cells of the anterior lobe.

Since acromegaly is ordinarily accompanied by a tumor which compresses and seriously compromises or may even destroy the normal hypophysial tissue, the continuous pathological overgrowth must be due to the secretory activity of the tumor itself. This assumption is strengthened by finding that the cells of the tumor contain the same granules that are present in the normal eosinophilic cells of the anterior lobe. The further assumption is therefore justifiable that these alpha-granules represent the secretory product that has to do with growth.

We are further struck by the fact that beta- (basophilic) granules are never found in hypophysial adenomas. It is true that basophilic adenomas of microscopical size have been reported by Erdheim and others, but we have never found, by the use of specific stains, beta-granules in the cells of any of the two hundred or more adenomas that have been carefully examined. Though adenomas are occasionally described as basophilic, this is probably due to the fact that the cells of the ordinary chromophobe adenoma stain somewhat more heavily with hematoxylin than with eosin. We have no clue as to the significance of the beta-granules, either from the study of adenomas or from any other source. Whether they are in any way related to the alpha-granules is a subject of dispute.

The chromophobe cells of the anterior lobe are supposed to be more embryonic in type than the chromophile cells and this supposition seems in accord with the fact that the chromophobe adenomas are almost always accompanied by symptoms of lowered hypophysial function and never by symptoms of overfunction. They appear, moreover, to be in general more rapidly growing than the eosinophilic adenomas.

While it is true that the cells of acromegalic adenomas contain alpha-granules, yet they have not the exact structure of normal eosinophilic cells and great numbers of them contain no granules. It is difficult to say whether these non-granular cells are embryonic chromophobe cells or eosinophilic cells which have lost their secretory activity. Kraus,[15] believing that certain non-granular cells of the normal anterior lobe are chromophile cells which have lost their granules, speaks of them as "*entgranulierten Zellen*." On the other

hand, as we have already noted, most of the chromophobe cells of the normal hypophysis appear to be of an undeveloped and more or less embryonic type.

The possibilities are several: (1) that the cells of the adenomas begin as chromophobe elements and subsequently acquire their acidophilic granules; (2) that they originate as alpha-cells and later lose their granules; or (3) that the cells in a given tumor, whether chromophile, intermediary or chromophobe, remain of the same type from the beginning to the end of the process.

Although we have occasionally had the opportunity of studying specimens from a given adenoma taken at different stages of the disease, we have never observed any essential change to have taken place in its cellular characteristics; as a matter of fact, surgical specimens are rarely secured from these tumors in other than the late stage of their development. We accordingly are shut off from employing this direct method of observing cytological alterations that may possibly occur in the tumor as its growth progresses, and are obliged consequently to turn to indirect evidence in discussing these hypotheses.

In accordance with the first hypothesis we would expect the patient to show primary signs of hypofunction on which evidences of acromegaly are subsequently grafted; if the clinical records are to be relied upon, this seems actually to have happened in two of our cases, but it must be exceedingly rare. The symptomatic converse, which would accord with the second hypothesis, namely to see signs of ultimate pituitary insufficiency in a patient primarily acromegalic, is, on the other hand, far from uncommon. But experience now teaches us that there are other patients with obvious dyspituitarism who show fugitive symptons of acromegaly from the beginning to the end of their disease.

It is in the adenomas of patients in this last category that the cells containing only a ring of fine alpha-granules in the periphery of the cytoplasm are to be found. And since these cells have a close resemblance to the young alpha-cells as they first appear in the embryo (Fig. 19) it is reasonable to suppose that they have never attained maturity and that they remain of this embryonic type throughout the course of the disease.

In further consideration of the second hypothesis it is interesting to consider the structure of the adenoma of those cases of well de-

veloped acromegaly, which at the time of operation have apparently passed into a phase of hypofunction (as evidenced by a low basal metabolic rate, hypotrichosis, etc.) in the absence of cystic degeneration, hemorrhage into the tumor or other destructive lesion which might account for the change in the clinical syndrome. One would expect from the general structure of the adenomas in these cases (*i.e.*, rounded cells with central hyalinization, crescentic excentric nuclei, numerous multinculeated cells, rare vascular sinuses, etc.) that all of the cells would prove to be crowded with alpha-granules. But as mentioned (*cf.* p. 551) among the exceptions to the rule that the more numerous the alpha-cells the more pronounced was the acromegaly, numerous cells are found in these transitional cases which are entirely free from granules. On the basis that these non-granular cells were probably once eosinophilic and now functionless (*entgranulierten Zellen* of Kraus), it is possible to understand the change of the clinical syndrome occurring in these cases.

These of necessity are purely theoretical considerations and when one recalls how long a time we have been in coming to an understanding of the nature of the adenomas of the thyroid gland which are far more accessible for study, impatience to get at the truth regarding the cytological evolution of the hypophysial adenomas may well be restrained. Meanwhile in concluding this report it is safe to say in:

Summary

That whereas a highly chromophilic type of adenoma, whose cells are heavily laden with alpha-granules, characterizes outspoken acromegalic hyperpituitarism, and whereas an adenoma of purely chromophobe type with non-granular cells is commonly associated with adult hypopituitarism, there is an intermediary group of cases in which traces of these opposed symptoms have apparently been present from the outset and which are associated with a histologically distinctive adenoma with cells of foetal type having sparse, peripherally disposed granules.

Since this intermediary syndrome is distinguishable clinically from the more common hypopituitary state by recognizable traces of hyperpituitarism, we find it convenient to refer to the disorder as *fugitive acromegaly*.

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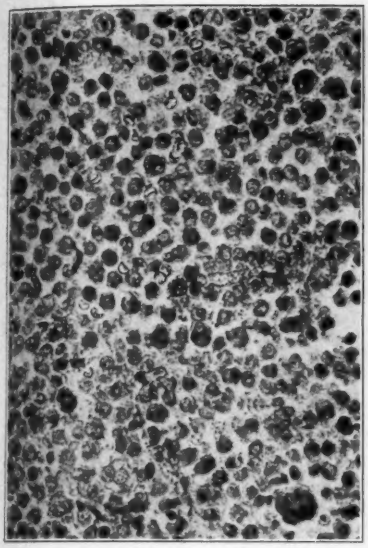
DESCRIPTION OF PLATES

PLATE 123

FIG. 1. Chromophile adenoma of Type I. (*a*) The cells of variable size, often multinuclear, lie in a loose, practically non-vascular mass (hematoxylin and eosin, × 300). (*b*) Practically every cell is packed with apha-granules (ethyl violet-orange G, × 850).

FIG. 2. Chromophile adenoma of Type II. (*a*) The tumor is more compact: one multinucleated cell is seen; the nuclei are more uniformly spherical and vesicular (hematoxylin and eosin, × 300). (*b*) Many cells contain abundant alpha-granules in the periphery of the cytoplasm (ethyl violet-orange G, × 850).



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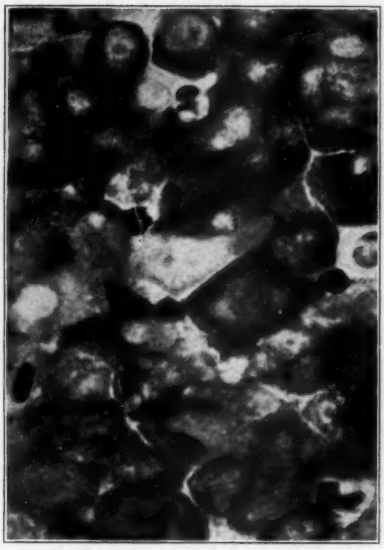

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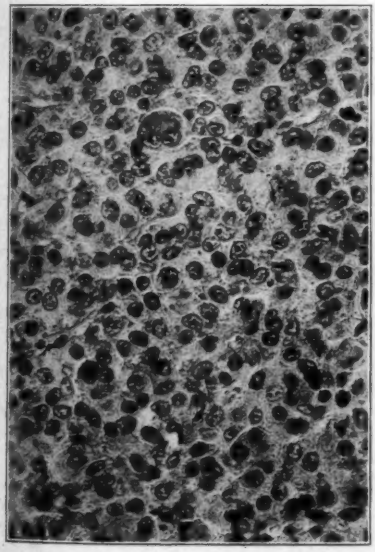

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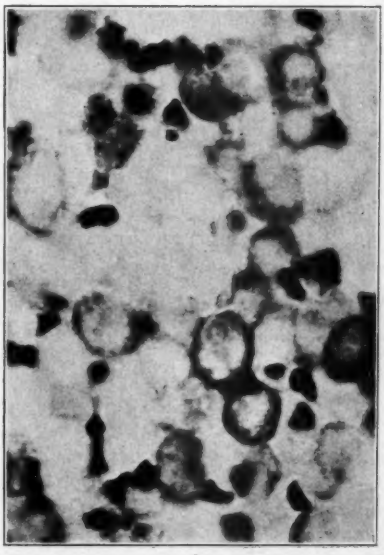


2 b

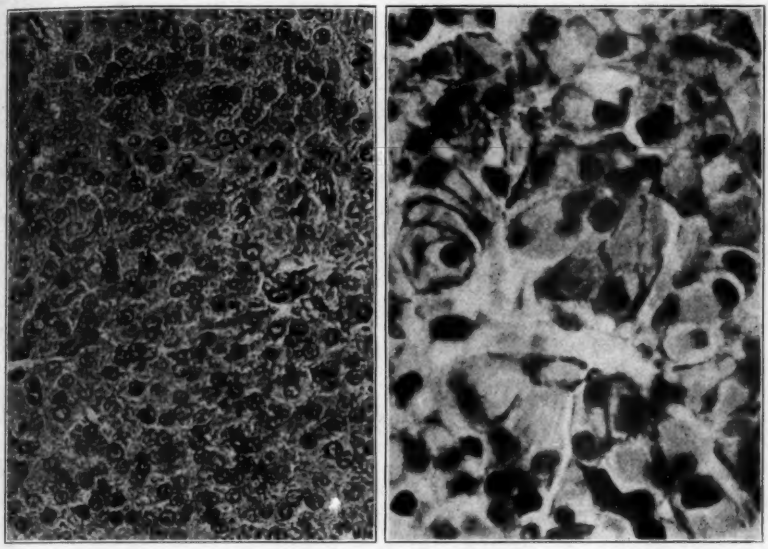
Bailey and Cushing

Adenomas in Acromegalic Dyspituitarism

PLATE 124

FIG. 3. Transitional adenoma of Type III. (*a*) The cells have vesicular nuclei with less abundant cytoplasm. No multinucleated cells. Few vascular sinuses. (Hematoxylin and eosin, × 300.) (*b*) Note the dark ring formed by alpha-granules in the peripheries of the cells (ethyl violet-orange G, × 850).

FIG. 4. Chromophobe adenoma of Type IV. (*a*) The tumor is similar to Type III though the nuclei are less consistently vesicular (hematoxylin and eosin, × 300). (*b*) No alpha-granules. Only the nucleoli and erythrocytes are stained (neutral ethyl violet-orange G, × 850).



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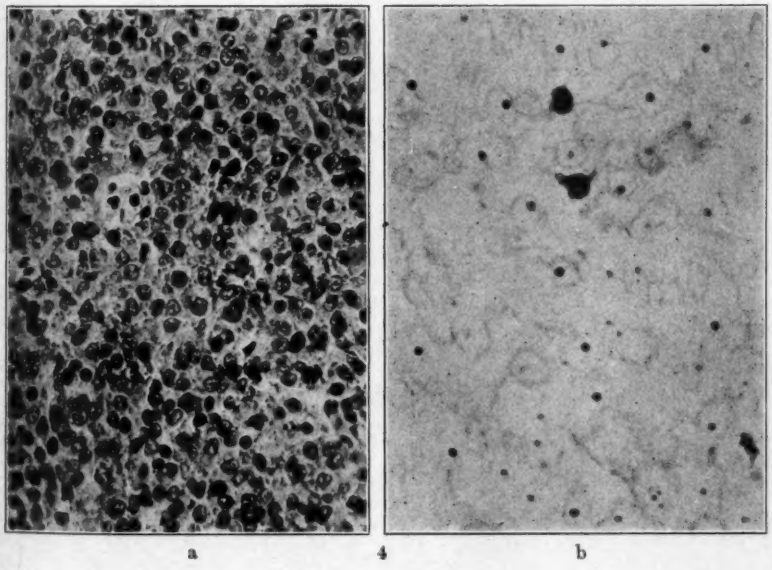

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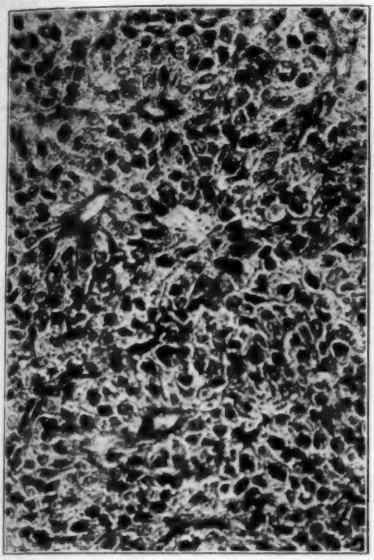
Bailey and Cushing Adenomas in Acromegalic Dyspituitarism

PLATE 125

FIG. 5. Chromophobe adenoma of Type V. The poorly defined cells show a tendency to radiate around numerous small vascular sinuses (methylene blue-eosin, × 300).

FIG. 6. Chromophobe adenoma of Type VI. The broad columns of cells whose nuclei are non-vesicular are separated by a stroma of connective tissue holding vascular sinuses (hematoxylin and eosin, × 300).

FIGS. 7 and 8. Case I. Note the large hand, thick lips and nose, coarse skin and prominent maxillary region.



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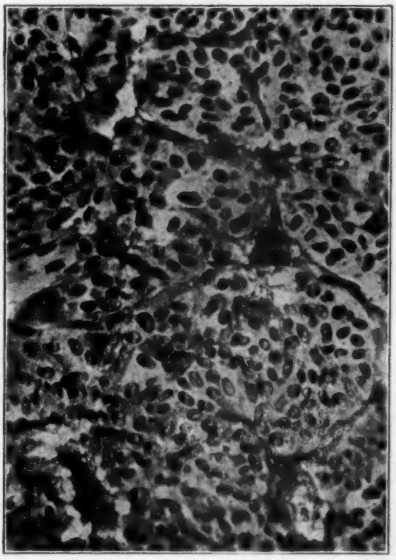

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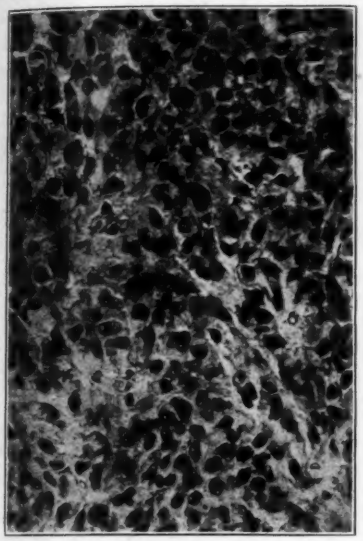
Bailey and Cushing

Adenomas in Acromegalic Dyspituitarism

PLATE 126

FIG. 9. Case I. Transitional adenoma of Type III. (*a*) (Hematoxylin and eosin, × 300.) The nuclei have a tendency to be vesicular. (*b*) (Neutral ethyl violet-orange G, × 850.) Two cells may be seen with a rim of alpha-granules.

FIGS. 10 and 11. Case II. Slight obesity with coarsening of the skin and features. Hand fairly normal.



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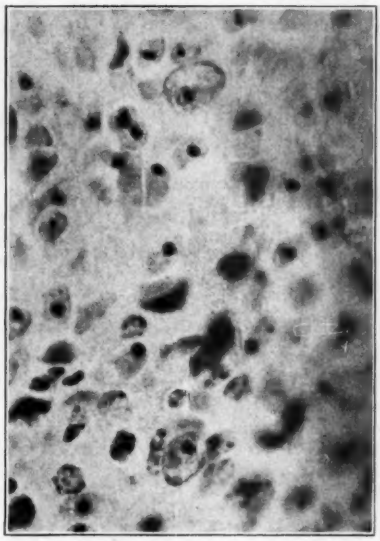

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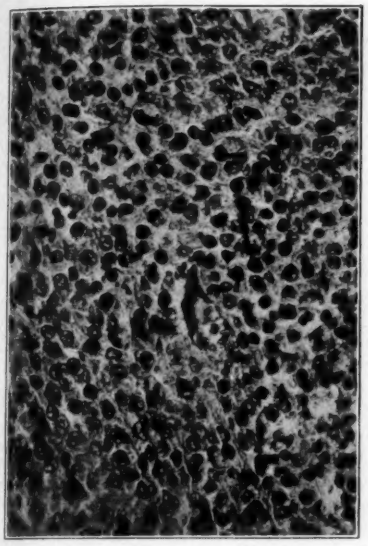
Adenomas in Acromegalic Dyspituitarism

PLATE 127

FIG. 12. Case II. Transitional adenoma of Type III. (*a*) (Hematoxylin and eosin, × 300.) Nuclei tend to be vesicular, vascular sinuses rare, no stroma. (*b*) (Neutral ethyl violet-orange G, × 850.) Shows a single cell containing a ring of alpha-granules in the periphery of its cytoplasm.

FIGS. 13 and 14. Case III. Large, heavy-set man, with obesity and traces of acromegaly shown by the X-ray films.



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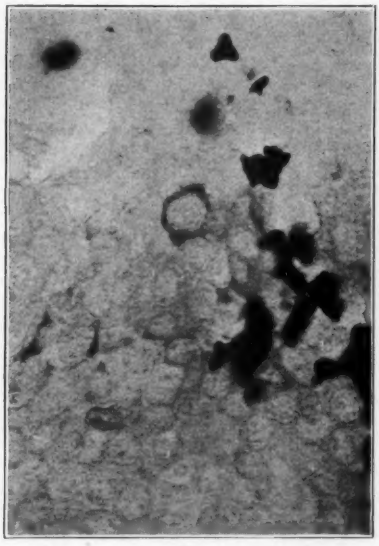

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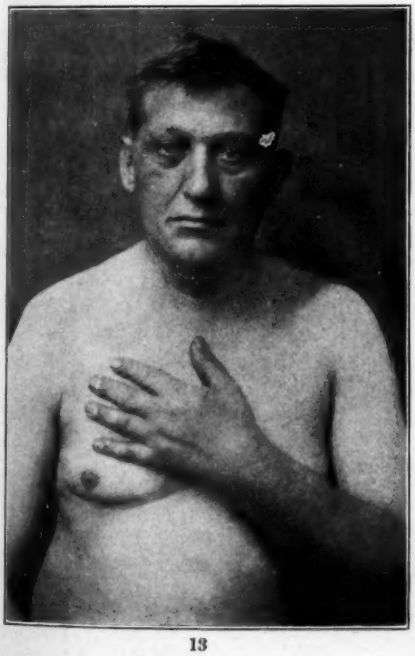

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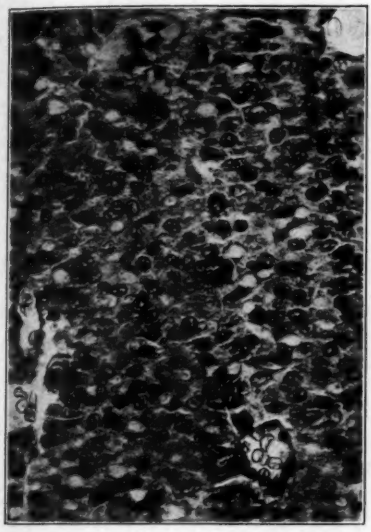
Bailey and Cushing

Adenomas in Acromegalic Dyspituitarism

PLATE 128

FIG. 15. Case III. Transitional adenoma of Type III. (*a*) (Hematoxylin and eosin, × 300.) Nuclei tend to be vesicular, sinuses rare, no stroma. (*b*) (Neutral ethyl violet-orange G, × 850.) One cell is seen, in the center, with a rim of alpha-granules.

FIGS. 16 and 17. Case IV. Dyspituitarism with thickening and puffiness of the subcutaneous tissue of the face and with glycosuria.



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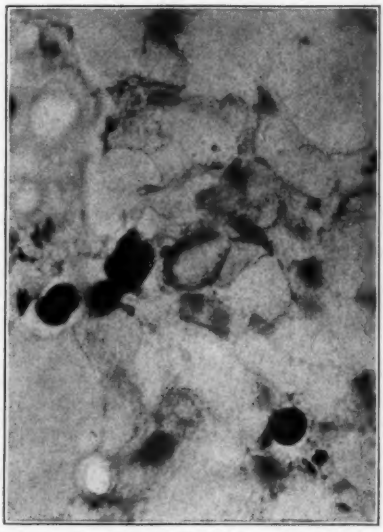

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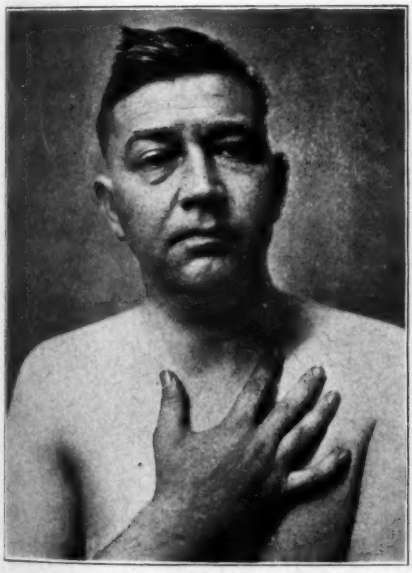

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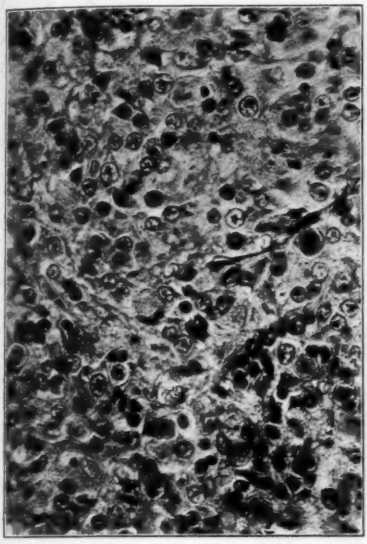
Bailey and Cushing

Adenomas in Acromegalic Dyspituitarism

PLATE 129

FIG. 18. Case IV. Transitional adenoma of Type III. (*a*) (Hematoxylin and eosin, × 300.) Nuclei are mostly vesicular, the vascular sinuses are rare, there is no stroma. (*b*) (Neutral ethyl violet-orange G, × 850.) Several cells are seen with a rim of alpha-granules.

FIG. 19. Hypophysis of new-born rat, showing the first appearance of alpha cells (neutral ethyl violet-orange G, × 850).



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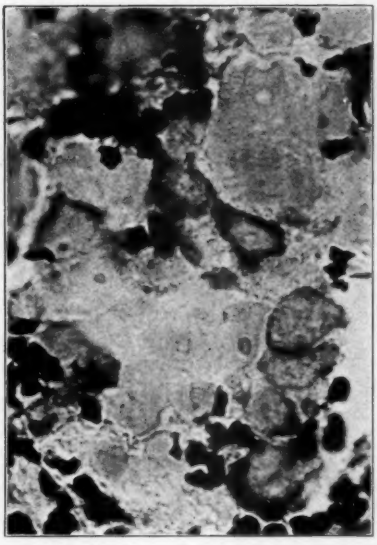

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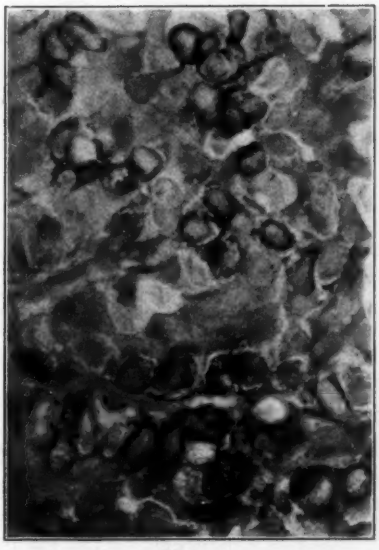

19

EXPERIMENTAL SUBCUTANEOUS RHEUMATIC NODULES*

B. J. CLAWSON, M.D.

(From the Department of Pathology, University of Minnesota, Minneapolis, Minn.)

The subcutaneous rheumatic nodule is a localized area of inflammation, mostly proliferative in character, found in the loose connective tissue beneath the skin in a limited number of cases of acute rheumatic fever. Its structure is similar to that found in the Aschoff nodule in the heart or in rheumatic inflammation in other parts of the body, as in the joints, galea aponeurotica, diaphragm, blood vessels, etc.

Apparently the first written account of these subcutaneous nodules was given by Hillier[1] in 1868. In 1875 Meynet[2] pointed out that these nodular areas bore a direct relationship to rheumatic fever. The nodules, according to Angel Money,[3] are less likely to occur in the extremely acute cases than in the chronic. Cheadle[4] seldom found them in acute rheumatic fever except in childhood.

Hirschsprung[5] in 1881 gave the first microscopic description of the nodules. He described them as consisting of connective tissue cells in different modifications. These cells varied in size and shape. Some were spindle-shaped and others were irregular. They contained one or more vesicular nuclei. These nuclei, he observed, were larger than those found in ordinary granulation tissue. He also noted that the ground substance between the cells consisted of a homogeneous material and that there was an increase in the number of blood vessels. In structure, the nodule resembled the tubercle, he thought, but did not have its typical appearance. He defined the nodule as being a localized area of inflammation with a tendency to undergo necrosis.

In 1881 Barlow and Warner[6] described the reaction in these subcutaneous rheumatic nodules as being similar to the reaction in the valves in acute rheumatic endocarditis. The same observation has recently been made by Swift.[7]

The presence of a proliferative endarteritis in these nodules was observed by Cavafy[8] in 1883. He noticed that the proliferating intima completely closed the vessel in some cases.

* Received for publication July 2, 1928.

Futcher[9] in his microscopic description of the subcutaneous rheumatic nodule states that it consists of fibrous tissue in various stages of development. In some nodules he found the cellular element to be made up of small round cells, fibroblasts, and polymorphonuclear leucocytes. He also noted giant cells, some of which contained as many as twenty-six nuclei. All stages were present from the young cellular nodules to the older fibrous ones. Some nodules even became calcified.

The most complete microscopic description of the subcutaneous rheumatic nodules was made by Frank[10] in 1912. He described them as being divided into a central and a peripheral portion. The center consisted of a homogeneous mass which stained intensely red with eosin, and by special staining he decided that this homogeneous material was fibrin. This fibrinous exudate extended out between the cells into the periphery. The periphery consisted of proliferative connective tissue which appeared partly as spindle cells and partly as epithelioid cells. Scattered among the proliferating cells a large number of leucocytes could often be seen. A variation in structure was noted in different nodules. This variation Frank considered to be due to different stages in the development of the same process, and he thought the primary reaction in the nodule was an exudation of polymorphonuclear leucocytes which was followed by a wandering in of round cells and by a proliferation of the surrounding connective tissue cells.

Swift[7] described the subcutaneous nodules as being made up of a conglomerate number of smaller nodules and as being similar in structure to the nodules found in the heart and other parts of the body. He observed that the center consisted of necrotic material and a small amount of fibrin.

There seems to be a general agreement by observers who have studied the subcutaneous rheumatic nodules that they consist of proliferating connective tissue cells and a cellular exudate of lymphocytes, plasma cells, and polymorphonuclear leucocytes in varying numbers and that in the center of most of the nodules there is a greater or less amount of homogeneous substance consisting of necrotic material and fibrin. There is also agreement that anatomically and etiologically the subcutaneous nodule is similar to the nodule in the heart described by Aschoff,[11] Geipel,[12] Coombs,[13] and others, and to the type of inflammation found in other parts of the

body in acute rheumatic fever. This type of inflammation has come to be considered by many observers a specific reaction to the rheumatic virus.

In this paper a microscopic study is made of human subcutaneous rheumatic nodules and of the nodules produced experimentally in the subcutaneous tissue in rabbits by injecting streptococci. The purpose is to compare the structure of a known rheumatic lesion with that of a lesion which has been produced experimentally to arrive at further conclusions concerning the causal relation of streptococci to rheumatic infections.

Human Subcutaneous Rheumatic Nodules

The human subcutaneous rheumatic nodule which is studied in this paper was removed from the subcutaneous tissue of a patient having acute rheumatic fever. The nodule was found, upon microscopic examination of serial sections of the entire nodule, to be made up of many smaller nodular areas of inflammation. The reaction observed in this nodule was chiefly proliferative in character, but in many parts exudates of polymorphonuclear leucocytes, lymphocytes, and what appeared to be fibrin were noted (Fig. 1). The proliferative cells varied in size, shape and in staining qualities. Some of the nuclei were vesicular in character and some were hyperchromatic. The cytoplasm also varied in its degree of taking the basic stains. Many of these cells were multinucleated. Some of the nodular areas showed more or less necrosis in the centers. There was an increase in the number of blood vessels, and proliferative endarteritis was noticed in the smaller vessels. Morphologically the reaction observed in this nodule was similar to that described by Hirschsprung, Futcher, Frank and others.

Experimental Subcutaneous Rheumatic Nodules

Nodules were produced in the subcutaneous tissues of rabbits by injecting different strains of streptococci in varying amounts and at different intervals. These nodules were removed and the microscopic structure was studied and compared with the structure of the human subcutaneous rheumatic nodule.

Five different strains of streptococci were used in the experiments. Two of these strains were isolated from the blood of patients having

acute rheumatic fever, one from the blood of a patient with subacute bacterial endocarditis, and two from pus from sinuses in cases of sinusitis. All of these organisms produced methemoglobin on the blood agar plate. Those strains isolated from the blood of patients with acute rheumatic or subacute bacterial endocarditis were less virulent to rabbits than the strains isolated from the sinuses.

Ten rabbits were injected intracutaneously and subcutaneously in many places with these organisms. Most of the animals had been previously injected with strains of streptococci intra-arterially through the left ventricle of the heart. Others had been previously injected subcutaneously in one area with a mixture of streptococci and agar. Just what part allergy may have played in the reaction in these experiments cannot be stated definitely.

The reaction found in the injected areas of the animals depended upon the virulence of the organism, the number of organisms injected, and the time of removal of the nodules after injection. The most virulent organisms tended to produce abscesses but in some cases typical proliferative nodules were produced. With the same organism the reaction varied according to the number of organisms injected. With a very few organisms proliferative nodules were produced, while with larger amounts abscesses resulted. With the same organism and approximately the same dose the nodules examined a day or two following the injection, as a rule, showed chiefly polymorphonuclear leucocytes, while in the nodules examined at from seven to fifteen days after the injection the chief reaction was proliferative in character. These findings are in agreement with Frank's conception of the pathogenesis of subcutaneous nodules, *i.e.*, the first reaction is an exudative one which is followed by a wandering in of lymphocytes and by a proliferation of the connective tissue cells. In all the rabbits injected some proliferative nodules were found, but such nodules were most frequent in the rabbits injected with small doses of the less virulent organisms. With the more virulent organisms there was a greater tendency for abscesses to develop.

In the proliferating nodules produced in rabbits many mononuclear and multinucleated cells with vesicular nuclei were observed (Figs. 2, 3 and 4). Some cells were large and irregular. Morphologically the cells found in these nodules appear similar to those in the human rheumatic subcutaneous nodules and in the human

Aschoff nodules in the heart. In some of these nodules, areas of slight necrosis were noticed. A varying number of polymorphonuclear leucocytes and lymphocytes were also present. There was an increase in the number of blood vessels and in some of these nodules there was an endarteritis.

It seems evident from these experiments that, by injecting streptococci into the subcutaneous tissues of rabbits, lesions can be produced which are morphologically similar to the nodules found in the subcutaneous tissue in cases of acute rheumatic fever. Since these experimental nodules occur obviously as a result of injecting streptococci, the probable conclusion is suggested that acute rheumatic fever and the type of inflammation associated with it are of streptococcic origin.

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DESCRIPTION OF PLATE

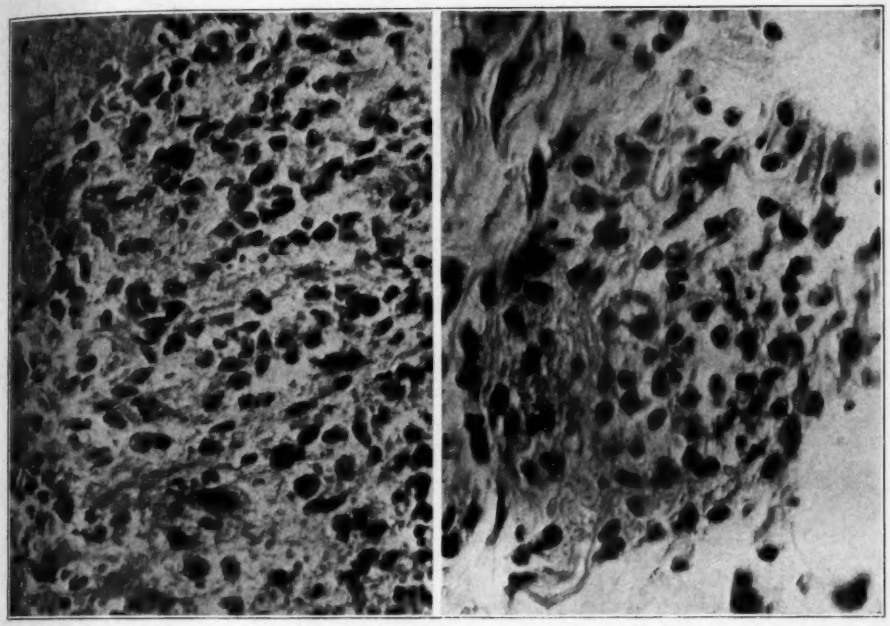
PLATE 130

FIG. 1. Human subcutaneous rheumatic nodule.

FIG. 2. Experimental nodule in the subcutaneous tissue of a rabbit.

FIG. 3. Experimental nodule in rabbit showing irregular proliferative cells.

FIG. 4. Experimental nodule in rabbit showing mononuclear and multinucleated proliferative cells.



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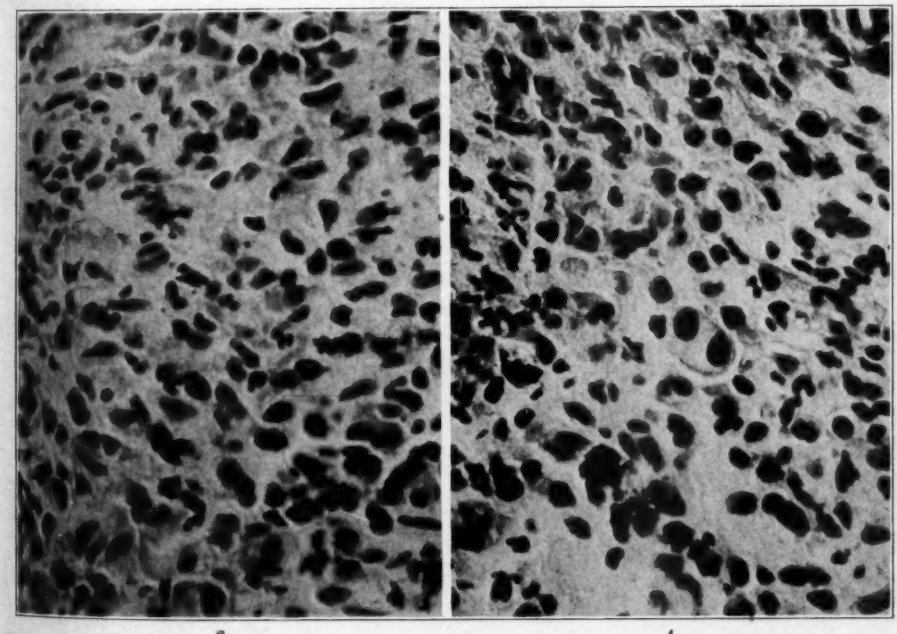

3 4

Clawson Experimental Subcutaneous Rheumatic Nodules

EXPERIMENTAL GLOMERULONEPHRITIS PRODUCED BY INTRARENAL TUBERCULIN REACTIONS*

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WITH THE TECHNICAL ASSISTANCE OF PAUL J. PATCHEN

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The tuberculin reaction, occurring when the specific protein of the tubercle bacillus comes into contact with the hypersensitive tissue of a tuberculous animal, illustrates several of the well known histological features of acute and chronic inflammation. Previous studies, of which this is an outgrowth, have shown that in a number of organs two broad phases of the reaction may conveniently be distinguished. These are (1) an acute exudative stage with degeneration or necrosis of local tissue, followed by (2) a stage of repair in which the products of the acute reaction are removed by absorption or proliferative organization of the exudate.

Both of these stages may be clearly seen in the familiar tuberculin skin reaction. They are still more conspicuous in the testis.[1] When a small amount of tuberculin is introduced into the testis of a tuberculous guinea pig, an initial acute exudative reaction occurs, with active hyperemia and escape of plasma and leucocytes into the interstitial tissue, and at the same time with severe injury to the cells of the seminiferous tubules, the spermatocytes being particularly affected. This reaction, which takes place within twenty-four hours, is followed by a period of absorption of the exudate and degenerated parenchymatous cells. At the end of a month the germinal cells, with the exception of the spermatogonia, are entirely absorbed, and the interstitial tissue is increased. The reaction is specific for the hypersensitive state, the introduction of tuberculin in the testis of the normal animal being without effect.

Tuberculin reactions of varying intensity may be produced in other organs. The results secured in the kidney have been found to

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be of particular interest, and seemed to us to warrant special investigation for a possible bearing upon the puzzling problem of spontaneous inflammation of the kidney in man.

That nephritis may at times occur as a manifestation of an allergic reaction has been many times suggested. This possible etiology has been particularly stressed by Longcope,[2] and supported by his observations on the effect of intravenous injection of foreign proteins into animals sensitized to these proteins. Changes developing in the kidneys resembled the lesions of nephritis in man.[3]

Rist and Leon-Kindberg [4] noted an allergic response in the kidneys of tuberculous dogs after intracardiac injection of tubercle bacilli. Hemorrhage and diffuse degeneration of the renal epithelium occurred. Ophüls [5] has advanced the view that allergic reactions may be at the basis of some of the glomerular forms of nephritis observed in man. Several investigators, including Ophüls, have stressed the possibility that an allergic element may be present in the inflammatory response of the glomeruli to the nephritis associated with subacute bacterial endocarditis. Thus an allergic ground for both degenerative and inflammatory lesions of the kidney has been suggested.

Experimental

Impressed by the severity of injury produced by tuberculin in the testis of a sensitive animal, we have attempted to produce analogous intrarenal tuberculin reactions. The first trial, injection of tuberculin preparations directly into the body of the kidney in guinea pigs, in a manner similar to that employed for testis inoculations, failed to yield results of significance. The attempt was then made to localize an inflammatory reaction to the glomeruli by arterial injection. Dogs, first selected for the purpose, proved to be unsuitable, because not sufficiently sensitive to tuberculin. Swine, which are sensitive to tuberculin following the artificial production of a mild tuberculosis, were next chosen. In the first experiments various preparations of tuberculin, and later dead tubercle bacilli, were injected with a 10 cc. glass syringe into the exposed renal artery under ether anesthesia. This procedure resulted in the production of scattered glomerular lesions of an early acute and later proliferative type.

In order to secure a more diffuse type of lesion a method similar to that recently reported by Miller and Apfelbach [6] was used.

These investigators perfused the kidneys of dogs with a suspension of carbon particles in the effort to produce glomerular obstruction, allowing sufficient time for the normal cycle of variation in the circulation to different parts to occur. In this way the majority of the glomeruli were reached.

The apparatus used in the experiments here reported is shown in Fig. 1. The tuberculin preparation employed was a protein, highly active in the tuberculin test, isolated from tuberculin by Dr. Seibert in a previous investigation.[7] This was dissolved in weak sodium hydrate and reprecipitated in a finely flocculent suspension by the addition of a few drops of N/10 hydrochloric acid. The suspension, which was faintly acid, was then added to a solution containing 1 per cent sodium citrate and 0.5 per cent sodium chloride. One hundred cc. of this preparation, containing 35–100 mg. of tuberculin protein, was introduced in the apparatus as shown in the figure, and pumped into the isolated renal artery of the experimental animal under ether anesthesia, the kidney being exposed extra-peritoneally by lumbar incision. By the use of the manometer shown, and observation of the point at which blood regurgitated through the needle into the glass syringe, it was possible to carry out the perfusion at a pressure 20–30 mm. Hg. above the blood pressure in the renal artery.

Six swine, two months old, were used in the final experiment here reported. Three of these were normal controls. Two had a localized lymph nodal tuberculosis produced by inoculation with 10 mg. of human type tubercle bacilli, and one a plastic peritoneal tuberculosis from intraperitoneal injection. Almost constantly the perfusion produced symptoms of shock in the tuberculous animals, while the normal pigs were unaffected. As a rule the left kidney was perfused first and the right kidney some weeks subsequently. In one instance a second perfusion of one kidney was made. The experiment was of a years duration. The anatomical effect of the injection was determined by biopsy, nephrectomy, or killing of the animal. The course of functional change was followed by analyses of the blood and simple examination of the urine. The blood analyses were made after the animals had been eighteen to twenty hours without food. Where blood analysis and operation were to be carried out on the same day, the blood for the chemical study was withdrawn first. Exhaustive study of the urine was not made, as trial soon showed

that the blood chemistry was a much more significant index of the condition of the animal.

The following protocols summarize the experiment:

PIG NO. I

Tuberculous Pig, Infected Subcutaneously on Oct. 20, 1926.

Dec. 7, 1926. Left kidney perfused with suspension of 40 mg. of tuberculin protein in 90 cc. salt-citrate solution.

Dec. 21, 1926. Right kidney perfused with suspension of 70 mg. of tuberculin protein in 100 cc. salt-citrate solution. Profound shock during operation.

March 13, 1927. Left kidney again perfused with suspension of 70 mg. of tuberculin protein in 100 cc. salt-citrate solution. Marked asthma one hour after operation. Pig drowsy and inactive throughout next day.

July 7, 1927. Right kidney removed by operation. Found to be simply a small hydronephrotic sac. No pulsation in renal artery.

Dec. 2, 1927. Pig killed with ether.

Description of Kidney (left); Gross: There is a moderate induration of the perirenal tissue. The capsule strips with some difficulty leaving a slightly pitted surface. The cortex is a little irregular, with a few depressed scars.

Microscopic: The majority of the glomeruli are not much changed. A moderate number of glomeruli show a small amount of hyaline scarring and mononuclear infiltration at the point of entrance and exit of the glomerular vessels. A few glomeruli are completely fibrosed, with atrophy of the corresponding tubules. In general the tubules are normal. The blood vessels are normal.

Blood Nitrogen

	12/7/26	12/21/26	1/16/27	3/13/27	3/28/27	6/4/27	7/7/27	9/5/27
Urea N, mg. per 100 cc. .	13.3	14.0	14.0	14.0	35.0	19.3	14.0	18.2
Total non-protein N, mg. per 100 cc.	40.0	30.0	41.7	35.0	62.4	45.5	37.6	41.7
Creatinine, mg. per 100 cc.	1.3	1.2	1.5	1.6	2.1	1.7	1.9	2.0

PIG NO. II

Non-Tuberculous Pig; Control to No. 1.

Nov. 24, 1926. Left kidney perfused with suspension of 40 mg. of tuberculin protein in 90 cc. salt-citrate solution.

Dec. 8, 1926. Right kidney perfused with suspension of 100 mg. of tuberculin protein in 100 cc. salt-citrate solution.

July 7, 1927. Right kidney removed. Found to be simply a small hydronephrotic sac.

Nov. 18, 1927. Pig killed with ether.

Description of Kidney (left); Gross: The kidney shows very little change from the normal. There is no induration of the perirenal tissue. The capsule strips easily, leaving a smooth, normal surface. The cortex is regular and the markings normal.

Microscopic: There is practically no change from normal. Rarely a small scar with a slight amount of mononuclear cellular infiltration is found.

Blood Nitrogen

	11/24/26	12/8/26	1/23/27	6/4/27	7/8/27	9/5/27
Urea N, mg. per 100 cc. . .	13.0	15.4	14.0	20.2	11.2	11.2
Total non-protein N, mg. per 100 cc.	30.0	41.6	37.0	41.7	35.5	38.5
Creatinine, mg. per 100 cc.	1.2	1.4	1.5	1.8	1.9	1.8

Pig No. III

Tuberculous Pig, Infected Intraperitoneally on Oct. 19, 1926.

Dec. 10, 1926. Left kidney perfused with 30 mg. of tuberculin protein suspended in 90 cc. salt-citrate solution.

Feb. 11, 1927. Right kidney perfused with 65 mg. of tuberculin protein suspended in 90 cc. salt-citrate solution.

Feb. 14, 1927. Pig killed with ether. Pig found to have a slight fibroplastic peritoneal tuberculosis with adhesions between loops of bowel and moderate tuberculous mesenteric lymphadenitis.

Description of Left Kidney; Gross: The kidney is smaller than the right. The perirenal tissue is indurated. The capsule strips with difficulty, leaving a slightly pitted surface. On section the cortex is found to be irregular in thickness, showing numerous areas of atrophy.

Microscopic: About 50 per cent of the glomeruli show change, consisting usually of an epithelial proliferation within the tuft. Frequently the damage is confined to single loop of the tuft. Such loops are often hyalinized and show a few polymorphonuclear leucocytes and pycnotic tissue nuclei. Epithelial organization of exudate

in the capsular space is occasionally seen. A small number of glomeruli are completely destroyed, being replaced by proliferating epithelium. No glomeruli are fibrotic. The tubules are atrophic in the neighborhood of the most severely damaged glomeruli. A few casts are present. The blood vessels are normal.

Description of Right Kidney; Gross: The kidney is swollen, mottled, and flecked with red dots of pin-point size. The capsule strips easily.

Microscopic: The majority of the glomeruli are changed. Many show a fibrinous exudate in the capsular space, in which polymorphonuclear leucocytes and large mononuclears are present. In nearly all glomeruli there is a marked proliferation of the capillary endothelial cells. Hyaline casts, often containing polymorphonuclear leucocytes, are abundant. Tubules not containing protein are collapsed. No vascular injury is seen, but there are a few small, subcapsular wedge-shaped infarcts.

Blood Nitrogen

	12/10/26	2/11/27*	2/14/27
Urea N, mg. per 100 cc.	10.5	18.2	34.3
Total non-protein N, mg. per 100 cc.	33.3	40.0	62.5
Creatinine, mg. per 100 cc.	1.3	1.6	2.0

* Pig had had access to some food within 12 hours preceding analysis.

PIG No. IV

Non-Tuberculous Pig, Control to No. III.

Dec. 14, 1926. Left kidney perfused with a suspension of 100 mg. of tuberculin protein in 100 cc. of salt-citrate solution.

Feb. 27, 1927. Right kidney perfused with 70 mg. of tuberculin protein in 100 cc. salt-citrate solution. The animal developed marked shock in the course of the operation, with much depression of the heart beat and respiration, but recovered in 20 minutes.

Mar. 2, 1927. Pig killed with ether.

Description of Left Kidney; Gross: There is no significant change except for some induration of the perirenal tissue.

Microscopic: Except for a few minute scars there are no changes. The glomeruli and tubules are normal. No changes are seen in the vascular system.

Description of Right Kidney; Gross: The kidney is profoundly changed. The perirenal tissue is edematous. The kidney is swollen. The capsule strips easily, leaving a mottled surface in which depressed white spots 2–3 mm. in diameter stand out sharply against a red background. There are some regions of superficial infarction. The cortical markings are in general obscured. The mucosa of the pelvis is swollen and hyperemic.

Microscopic: The glomeruli in all sections are much changed. Endothelial proliferation within the tuft is pronounced. Exudation of fibrin and leucocytes is seen in the capsular space in many instances. Practically all of the convoluted tubules contain hyaline casts. There are numerous small wedge-shaped regions just beneath the capsule in which both glomeruli and tubules are in a state of coagulation necrosis. Large vessels containing thrombi are not found, but some thrombi are seen in the venules.

Blood Nitrogen

	12/14/26	2/27/27	3/2/27
Urea N, mg. per 100 cc.	12.6	9.8	9.8
Total non-protein N, mg. per 100 cc.	31.2	25.0	27.8
Creatinine, mg. per 100 cc.	1.3	1.3	1.5

PIG NO. V

Tuberculous Pig, Infected Subcutaneously, on Oct. 19, 1926.

Jan. 16, 1927. Left kidney perfused with a suspension of 100 mg. of tuberculin protein in 100 cc. salt-citrate solution. Pig had asthma for one hour after operation, but was in good condition next day.

April 1, 1927. Right kidney perfused with a suspension of 50 mg. of tuberculin protein in salt-citrate solution. In the course of the operation the pig developed profound asthma, so that it was necessary to stop the perfusion before the intended amount was injected.

April 5, 1927. The wound over the right kidney was reopened. The kidney was found to be pale but flecked with bright red spots. A piece was removed for examination.

Microscopic Examination: The glomeruli are enlarged, and the capsular space is distended. There is, however, no exudate of fibrin or leucocytes within the capsular space. Many of the cells of the tuft (epithelial?) show marked vacuolization. There is some proliferation of the capillary endothelium, and a good many polymorphonuclear leucocytes are present within the loops of the tufts. Many of the convoluted tubules are much distended and contain dense

hyaline or leucocytic casts. In many regions there is much diffuse leucocytic infiltration of the interstitial tissue.

Jan. 19, 1928. Pig killed with ether.

Description of Left Kidney; Gross: The perirenal fat is more densely adherent to the kidney capsule than normally. The renal capsule strips with great difficulty leaving a surface showing many minute depressions. The cortex is slightly decreased in thickness, and the cortical markings are somewhat obscured.

Microscopic: Except for small subcapsular scars there are no conspicuous changes. The glomerular tufts and capsules are for the most part normal, although occasionally there is some thickening and slight hyalinization of the tissue at the point of vascular attachment. The tubules are in general quite normal, as are the vessels and interstitial tissue.

Description of Right Kidney; Gross: Resembles the left, except that the capsule strips more readily, leaving a smoother surface, and that the cortex is wider.

Microscopic: Sections removed from all parts of the kidney, including the regions adjoining the biopsy site of April 5, show slight but definite change. The chief abnormality is a proliferation of elongated cells at the site of the vascular attachment of the tuft. The nuclei of the cells stain densely, and the cytoplasm is abundant and hyaline. Distal to this region the tuft is usually normal. Occasional patches of endothelial proliferation are seen, and such areas frequently contain pycnotic polymorphonuclear leucocytes. A very few glomeruli are fibrosed. In general the tubules are normal.

Blood Nitrogen

	1/16/27	4/5/27	4/12/27*	6/4/27	9/5/27
Urea N, mg. per 100 cc.	10.5	25.2	30.1	16.0	15.4
Total non-protein N, mg. per 100 cc.	34.5	55.5	69.0	38.5	37.0
Creatinine, mg. per 100 cc.	1.3	1.6	1.6	1.6	1.6

* Following day of heavy meat diet.

Pig No. VI

Non-Tuberculous Pig, Control to No. V.

Jan. 23, 1927. Left kidney perfused with a suspension of 100 mg. of tuberculin protein in 100 cc. salt-citrate solution. Marked blanching of kidney. No symptoms of shock.

April 26, 1927. Right kidney injected with 40 mg. of tuberculin protein in 100 cc. salt-citrate solution.

April 30, 1927. The wound over the right kidney was reopened, and a piece of tissue removed for examination.

Microscopic: Except for the presence of a very few hyaline casts the renal tissue was normal. The inflammatory picture seen in the kidney of Pig No. V was entirely lacking.

Feb. 2, 1928. Pig killed with ether.

Description of Kidneys; Gross: The kidneys are unequal in size, the left being smaller. Otherwise they are quite similar. In each case the capsule strips easily, leaving a smooth surface. The smaller kidney shows no scarring. The cortex is of normal width in each case, and the vessels are normal.

Microscopic: No significant changes are seen in either kidney. The right kidney shows a few glomeruli with slight scarring at the point of vascular attachment, similar to that seen in the right kidney of Pig No. V, but on a less extensive scale. A moderate number of the tubules in association with such glomeruli contain hyaline casts. Otherwise the tubules are normal.

Blood Nitrogen

	1/23/27	4/26/27	4/30/27	5/4/27*	6/4/27	9/5/27
Urea N, mg. per 100 cc. . .	14.7	13.3	14.0	16.1	17.9	11.9
Total non-protein N, mg. per 100 cc.	34.5	33.3	37.0	38.5	47.5	35.0
Creatinine, mg. per 100 cc.	1.5	1.6	1.6	1.8	1.8	1.8

* Following day of heavy meat diet.

Discussion of Protocols for Pigs I and II

In this experiment no view of the kidney was obtained in the acute stage of the reaction, or the period of recovery. Accordingly the only information available on the immediate or early effect of the tuberculin injections is the record of the state of the animals

at the time of the injection and the condition of the blood subsequent to perfusion of the second kidney.

It is significant that no immediate reaction followed the injections of tuberculin protein into the normal pig, while in the case of two of the injections in the tuberculous pig general symptoms referable to the tuberculin perfusion occurred. The first injection was perhaps too small to bring them out. We originally intended to give 100 mg. of the protein on the perfusion of the second kidney, but the animal was in a state of such serious depression after the introduction of 70 mg. that it was necessary to stop the injection. This immediate or early reaction to tuberculin, in contradistinction to the late, well recognized, typical "tuberculin reaction," has been repeatedly observed by others, and is considered by Zinsser [8] to be a true anaphylactic response. It is manifested on intradermic injection within twenty minutes, by a reddening which soon passes away, while the true tuberculin reaction in the skin develops only after some hours and persists for a day or more.

The blood chemistry yielded information of great value on the state of renal excretion in the course of the experiment. At no time was there an appreciable retention of nitrogen in the blood of the non-tuberculous animal. In the tuberculous pig, on the other hand, a definite increase in the urea and total non-protein nitrogen of the blood occurred after the second injection of the second kidney. The first injection had apparently not been effective; the injection, as we have noted, was relatively small and had not produced a general reaction. From the results of later experiments in which biopsies were made in the acute stage of the reaction (Pigs V and VI) or the animals were killed two months after the injection (Pigs III and IV), we have reason to believe that on March 28, when the blood nitrogen of Pig No. I was high, the left kidney was in a state of acute reaction, or just recovering from it, while the right kidney was in a state of repair after the operation of December 21 (or out of commission entirely, as suggested by the postmortem finding). The kidneys of the non-tuberculous pig, on the other hand, as we may assume from later experiments, were at no time inflamed, and excretion proceeded normally.

The total destruction of the right kidney in each animal was surprising, and probably without specific significance. Vascular occlusion apparently developed in both the tuberculous and non-tuber-

culous pigs, an accident which did not occur in any of the other four pigs of this series or in seven other swine used in preliminary experiments where injection by syringe was practised.

The relative insignificance of the renal lesions noted at the postmortem examination of these animals approximately a year after the first injections, is of the utmost importance. Without the more carefully controlled experiment on Pigs No. V and VI its meaning would not be clear. In the light of the later experiment, however, we have reason to believe that in Pig No. I an initial acute inflammation occurred, followed by such nearly perfect repair that only minute scarring was left as evidence of the previous damage. The youth of the pigs at the beginning of the experiments may have been a factor in this recovery. In the period while the pigs grew two or three hundred per cent in size, the glomeruli developed correspondingly.

Discussion of Protocols for Pigs III and IV

In this experiment the necropsy afforded an examination of one kidney three days after the injection of tuberculin, and of the other kidney two months after its corresponding treatment.

In the tuberculous animal both left and right kidneys disclosed a state of inflammation. In the right kidney, which had been perfused with tuberculin protein three days before the pig was killed, and one hundred and sixteen days after the animal had been made tuberculous by intraperitoneal injection with tubercle bacilli, acute exudative and early proliferative glomerular reactions were seen. The exudative phase is illustrated in Fig. 2. In all respects it was similar in type to that occurring in tuberculin reactions in the skin or testis. Its wide distribution in this case justified the designation of the lesion produced as an acute glomerulonephritis.

The left kidney, injected sixty-five days before the pig was killed, and fifty-two days after the pig had been made tuberculous, was found at necropsy to be in the stage of repair after an acute inflammation. The essential lesions were glomerular in location and proliferative in character. Both tuft and capsule took part in the latter process. Epithelial and endothelial organization of exudate, the remains of which could still be seen, was evident. Fig. 3 shows an epithelial crescent, and Figs. 4, 5, and 6 proliferations of tuft or

capsule. Fig. 6 illustrates the atrophy of those tubules dependent for their nourishment on the glomeruli involved.

Many of the glomerular lesions in this case were indistinguishable from those commonly described in subacute glomerulonephritis. The involvement was not quite so extensive, however, as that considered pathognomonic for this condition. As, moreover, the term subacute glomerulonephritis is usually reserved for a progressive lesion, while the lesion in this case was reparative, and probably presaged healing (judging from the experience of Pig No. V), it is perhaps better to designate the lesion in Pig No. III simply as a proliferative glomerulonephritis.

In general there was a good deal of similarity in the glomerular lesions in this animal to those described and illustrated by Bell, Clawson and Hartzell [9] after intravenous injection of streptococci into monkeys, and by Duval and Hibbard [10] following injection of streptococcus toxin into rabbits. It is noteworthy that the first-named series of investigators made repeated injections of streptococci, and that in the later injections a systemic reaction occurred. It seems probable to us that a tissue sensitization to the injected substance may have played a part in their experiments as well as in ours.

The blood nitrogen in the case of Pig No. III showed a noteworthy rise following the injection of the second kidney. It may be assumed from the normal figure obtained for the blood taken before the operation, that as long as one kidney was uninjured, no retention occurred. All the experience of this series has supported this view.

The left kidney of the control, non-tuberculous pig (No. IV), which had received an even larger dose of the tuberculin protein than No. III, was found seventy-eight days after the perfusion to be practically normal. Obviously it had not passed through the stage of acute inflammation seen in Pig No. III.

The right kidney, on the other hand, was found profoundly injured three days after its injection. It will be noted that this pig had suffered from severe shock at the time of the operation, of that character which we had previously interpreted as anaphylactic. By analogous reasoning it might be assumed that the anatomic changes found were of the same nature, *i e.*, that the marked injury of the kidney was virtually an Arthus phenomenon, following sensitization by the first injection seventy-five days before. This is the best

explanation we have to offer. Against it is the fact that only this once in all the series did this picture occur. Vascular injury was certainly responsible for part of the process but a good deal of it seemed truly inflammatory in character.

In this pig, analysis of the blood at no time showed nitrogen retention. This is to be associated with the fact that, unlike the situation in Pig No. III, one kidney was intact throughout the experiment, as proved by postmortem examination.

Discussion of Protocols for Pigs V and VI

In this experiment a biopsy permitted a view of the kidneys of the two animals four days after the injection of tuberculin, and the postmortem examination nine months later afforded a comparison with the biopsy specimen.

In this case again it is noteworthy that the tuberculous pig suffered from asthma and shock at the time of each operation, while no such effect was observed in the non-tuberculous pig. The biopsy specimen from the tuberculous pig disclosed an acute nephritis, not as definitely glomerular in character as that seen in Pig No. III, but fully as severe and likewise as diffuse (Figs. 7 and 8). The biopsy specimen from the non-tuberculous pig, which had received the same treatment with tuberculin, showed no inflammatory reaction whatsoever.

As the protocols show, the kidneys of the tuberculous as well as the non-tuberculous animal one year after the first injection and nine months after the second, disclosed only minimal change from the normal. The histological picture at the end of the experiment was quite the same as that seen in Pig No. I. Fig. 9 illustrates the slight amount of persisting change in the tuberculous pig. Nevertheless we know that this kidney had at one time been in a state of diffuse, violent acute inflammation, and while no biopsy had been performed on the other kidney, there is every reason to believe that it was in the same state of acute inflammation following its injection.

The blood chemistry paralleled the anatomic injury. Following the tuberculin reaction in the second kidney, a definite retention of nitrogen, not present after the injection of the one kidney only, developed. This was accentuated by placing the animal on a heavy meat diet. Later, complete restoration of function occurred, as

shown by a return of the non-protein nitrogen of the blood to a normal figure. This is presumably to be correlated with the morphological recovery observed. In the non-tuberculous animal, in which the biopsy after tuberculin injection showed no appreciable change from the normal, the blood nitrogen remained normal throughout the course of the experiment.

Summary and Conclusions

The experiments described above demonstrate that, by the injection of tuberculin into the kidneys of swine made sensitive to this substance by the presence of a mild tuberculosis, it is possible to produce a diffuse inflammation of the kidneys that may properly be considered an acute glomerulonephritis. Moreover this effect is the result of a true tuberculin reaction, as it does not occur following the perfusion of the kidney of a non-tuberculous animal with tuberculin. We may therefore add the intrarenal tuberculin reaction to the intradermic, conjunctival, intratesticular and other anatomical varieties of tuberculin reaction previously described.

The immediate reaction in the kidney is followed by a subsidence of acute manifestations, and absorption and proliferative organization of the exudate. Many of the glomeruli in this stage show the epithelial and endothelial stimulation and growth seen in a late "acute proliferative glomerulonephritis," or in a subacute glomerulonephritis. True epithelial crescents are occasionally seen. Atrophy of the tubules associated with the more severely injured glomeruli occurs.

When the injury is bilateral, a moderate nitrogen retention develops. In the experiments recorded here, this was seen regularly in the tuberculous pigs which had been subjected to renal tuberculin perfusion, and never in the non-tuberculous animals similarly treated.

With the passage of several months following the intrarenal, or more specifically intraglomerular tuberculin reaction, almost complete restoration to an anatomical normal occurs, at least if very young pigs are used, as was the case in this experiment. As the animals grow and the kidneys develop to normal bulk, the glomeruli increase in size correspondingly. The only persisting signs of the former acute inflammation are small hyaline areas which occasion-

ally contain old pycnotic polymorphonuclear leucocytes. These are usually located at the point of vascular attachment of the tuft and capsule. Simultaneously with this anatomical healing, complete functional recovery also occurs, shown by a disappearance of nitrogen retention and return of the blood to normal.

The question naturally arises whether or not the artificial production of the nephritis here described has any relation to the spontaneous development of nephritis in man. It will be recalled that the majority of adult human beings in urban groups are sensitive to tuberculin, and therefore carry within themselves the potentiality for the type of injury herein recorded. It is conceivable that during the period of activity of a tuberculous focus, tubercle bacillus protein could be taken up, as by phagocytic cells, from the débris of the lesion and excite changes of an allergic nature at a distant point such as a glomerulus of a kidney. It is unlikely, however, that any such dosage as used in the experiments described could be so absorbed. It is possible, on the other hand, that occasional and scattered glomerular lesions could arise in this way. However, it must be remembered that the tuberculin reaction is only one of a type. A considerable number of analogous forms of sensitization exist. While such reactions are in general protective against the spread of the infection concerned, the act of protection itself may be destructive to the local tissue, and no initial distinction is made in the reaction to live bacteria or their dead specific protein. These facts, together with the experiments reported here, justify further work in this field.

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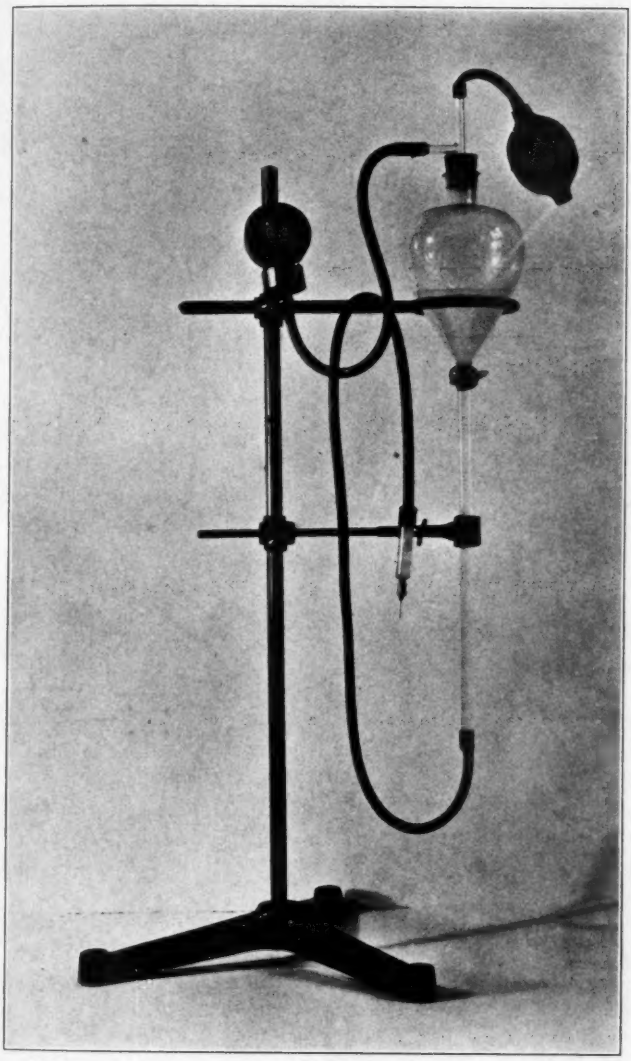
DESCRIPTION OF PLATES

PLATE 131

FIG. 1. Apparatus used for tuberculin perfusions. The glass bulb contains a suspension of tuberculin protein.

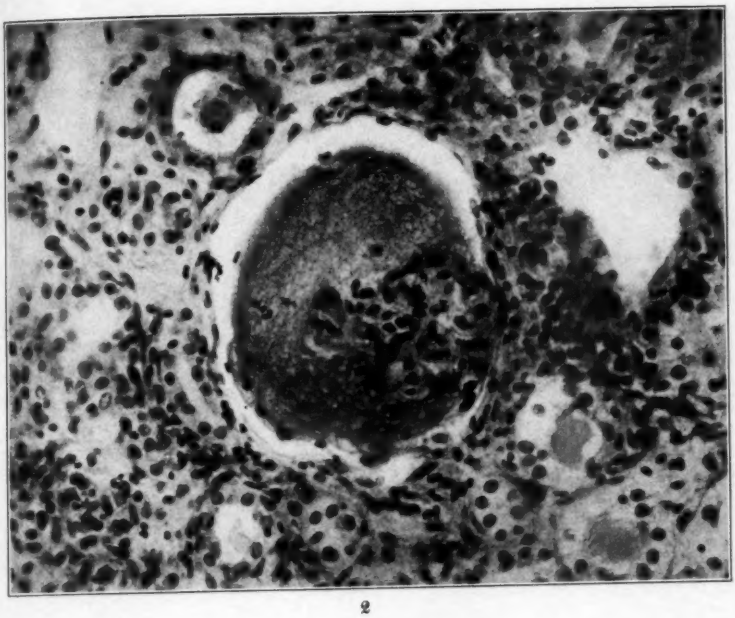


1

PLATE 132

FIG. 2. Acute exudative (tuberculin) reaction in glomerulus. From Pig No. III (tuberculous pig) three days after injection of tuberculin protein into right renal artery. × 300.

FIGS. 3. Proliferative lesions in glomeruli. From Pig No. III (tuberculous pig) sixty-five days after injection of tuberculin into left renal artery. × 200.



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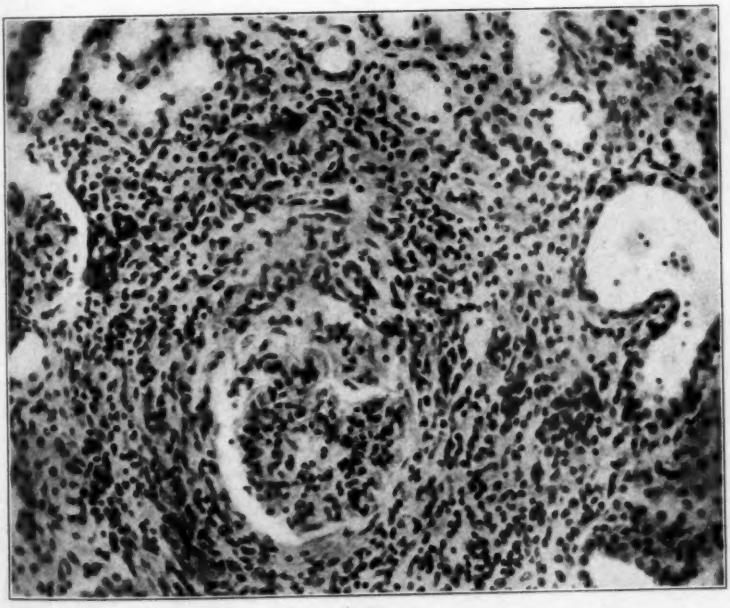
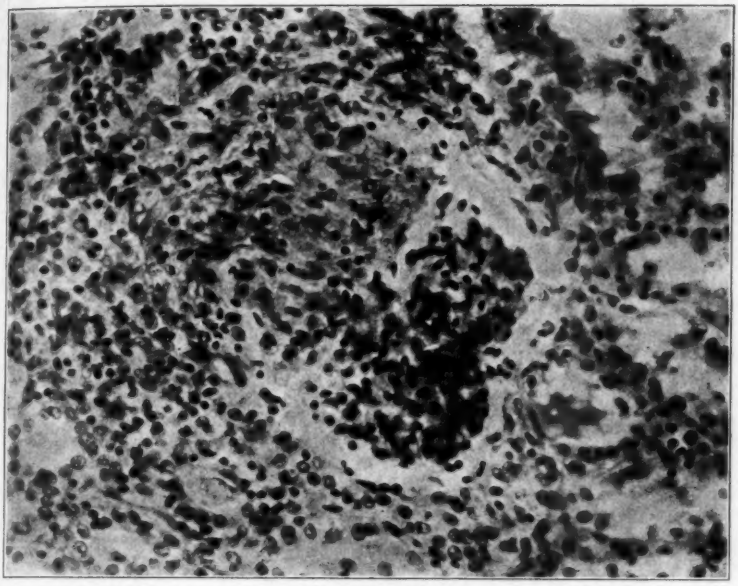


3

Long and Finner Experimental Glomerulonephritis

PLATE 133

FIGS. 4 and 5. Proliferative lesions in glomeruli. From Pig No. III (tuberculous pig) sixty-five days after injection of tuberculin into left renal artery. Fig. 4, × 300. Fig. 5, × 250.



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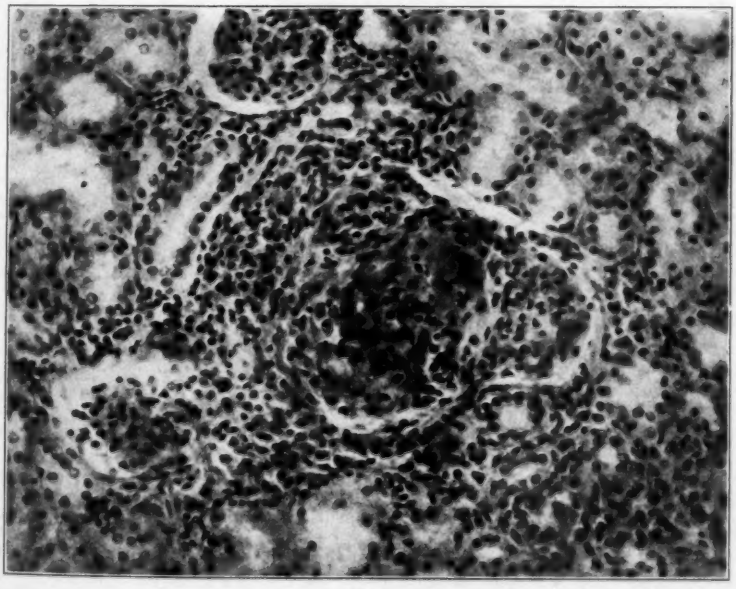


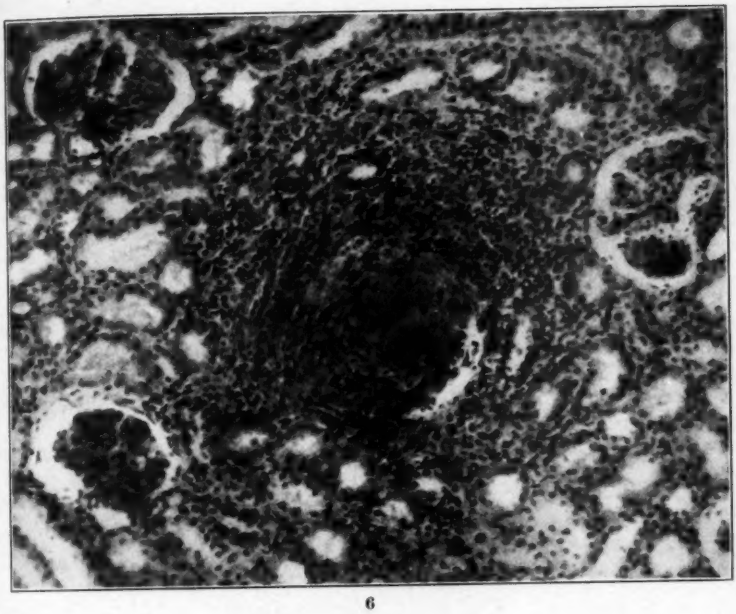
5

Long and Finner Experimental Glomerulonephritis

PLATE 134

FIG. 6. Same as Figs. 3, 4, and 5. × 170.

FIG. 7. High power view of renal lesion in Pig No. V (tuberculous pig) four days after injection with tuberculin-protein (biopsy). Note glomerular hemorrhage, increased cellularity of tuft, and blood and leucocytes in tubules. × 300.



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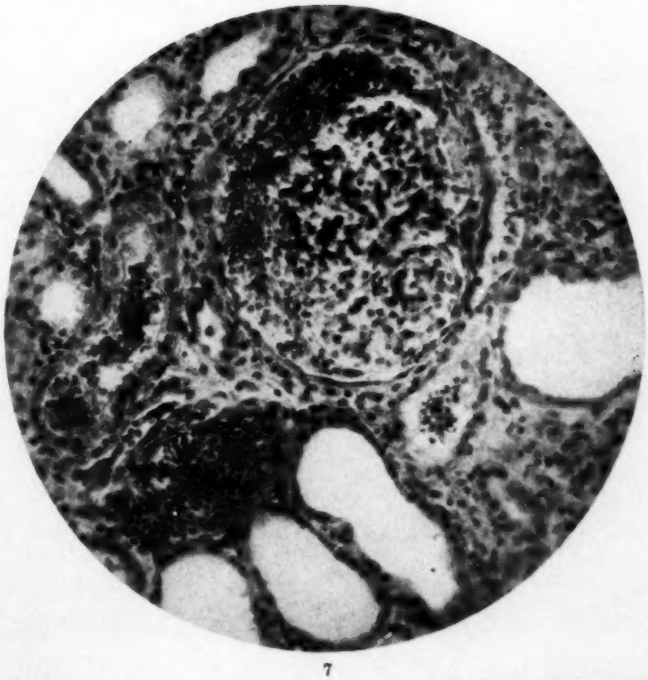

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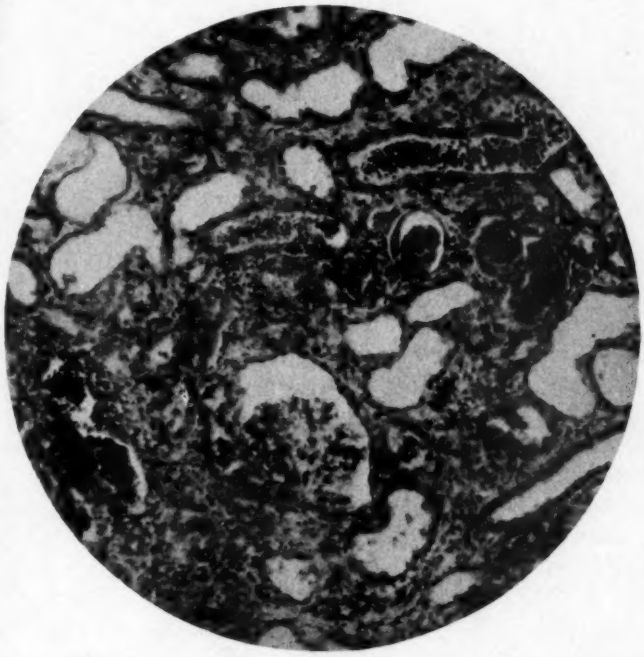
PLATE 135

FIG. 8. Same as Fig. 7. Note hyaline and leucocytic casts and distension of tubules. × 150.

FIG. 9. Slight persisting change in glomerulus nine and one-half months after injection of tuberculin protein. From Pig No. V (tuberculous pig) and from the same region of the kidney as the biopsy specimen illustrated in Figs. 7 and 8. Note the small amount of hyaline scarring at the point of vascular attachment of the tuft. The rest of the glomerulus and the tuft are normal. × 300.



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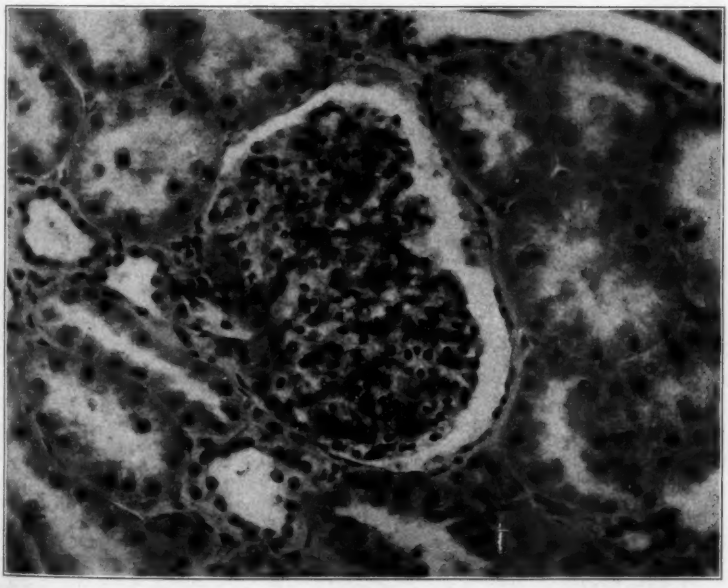

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Long and Finner Experimental Glomerulonephritis

THE PHAGOCYTIC ACTIVITY OF VASCULAR ENDOTHELIUM OF GRANULATION TISSUE *

F. A. McJunkin

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Much opposition has arisen to the view that the vascular endothelium may give rise to wandering phagocytic cells, and those sponsoring this opposition are inclined to the idea that the vascular endothelial cell is a highly differentiated element functioning only as a lining for the vessels. In an investigation including a study of the endothelium of the pulmonary capillaries Gardner and Smith [1] state: "Even after preliminary irritation with India ink these cells (the capillary endothelium) have failed to exhibit evidence of multiplication or of specific staining." Stilwell [2] ascribes to the endothelium a mere passive rôle in the handling of particulate matter. He thinks that carbon particles pass from the circulation through intact endothelium and are taken up by perivascular cells. Maximow [3] admits that the Kupffer cells of the liver are phagocytic and are active in storing colloidal dyes but he differentiates sharply such "histiocytic littoral" phagocytes from the ordinary endothelium lining capillaries. Foot [4] in a recent paper has abandoned his former opinion that the vascular endothelium is active in the production of phagocytes. He also apparently is inclined to the view that cells, such as the Kupffer cells of the liver, are anchored monocytes and not true endothelial cells. Sabin, Doan, and Cunningham [5] by supravital staining distinguish two types of connective tissue phagocytes, the monocyte and the clasmatocyte, which is a wandering endothelial leukocyte.

In my initial phagocytic experiments with suspended carbon, I [6] did not examine newly formed endothelium such as the vascular sprouts of granulation tissue. At this time, however, it was determined that stimulated endothelium, not only of the liver sinusoids but of the capillaries of other organs (heart), may ingest particles of carbon. I cannot subscribe to the expression of Stilwell that the incorporation of such particulate matter is passive. All carbon

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particles removed from the circulation do not pass passively from the endothelial-lined tube through cell interspaces, although some of them certainly do this, but a part of the microscopic granules are actively phagocyted into the cytoplasm of the endothelial cells. The Kupffer cells of the liver are continuously called upon to remove particulate matter from the blood stream and have for the most part a more abundant cytoplasm than the endothelium of capillaries elsewhere, but structurally these active cells are not only continuous with the capillary endothelium of the vessels emerging from the periportal connective tissue of normal liver but in the repair of wounds of the liver these sinusoids are continuous with the capillaries of granulation tissue. It was, and is now, my opinion that the usual, flat, inactive endothelial cell, functioning as a vascular lining, is non-phagocytic; but under suitable stimulus it may enlarge, undergo mitosis and become actively phagocytic. That the chief function of the blood vascular endothelium is to afford a smooth vessel lining is perfectly obvious but there appears to be a basis for the opinion that the capillary endothelium of certain normal organs may ingest particulate matter and so remove it from the circulation. Likewise under influence of a sufficient stimulus the usual type of endothelium elsewhere may assume this phagocytic rôle.

Methods and Results

The experiments were planned to determine the phagocytic possibilities of the endothelium of vascular sprouts in granulation tissue. The presumption was that some of the cells would become separated to give rise locally to free phagocytes provided the lining cells of the new vessels were found to be phagocytic. Proof of such local origin of free phagocytes was not included in the scope of the experiments. Carbon was used to test the phagocytic property of the cells. The granulation tissue was produced in some animals by the subcutaneous injection of large quantities of India ink and in others by the injection of suspended casein. Following the injection into the groin of 2 cc. of India ink (Higgins) the carbon tends at first to be encapsulated by the formation of vascularized connective tissue at its periphery. Some of the carbon at once passes to the regional lymph nodes where it marks some of the reticulo-endothelial cells.[6] In an examination of many blocks of such encapsulated carbon it was only

in an occasional section that the endothelium of the new vessels was found to be marked with carbon. When such a vessel was located the lining endothelial cells usually contained much carbon (Fig. 1). Owing to this irregular distribution the inference was drawn that the new vessels come into contact with the carbon but the particles do not enter their lumina and are not taken up by the endothelium. Ink may have entered, through mechanical rupture, the lumina of the vessels in which the endothelium was found to contain ink particles. A wider distribution of ink in the vessels was obtained by introducing the ink directly into the vascular system.

Experiment A: Using a syringe fitted with a large needle, half-grown rats were injected subcutaneously in the groin and axilla with 1 cc. of a sterile thick suspension of casein. After nine to twenty-one days the animals were etherized and 1 cc. of India ink injected into the heart. After five minutes the rats were chloroformed and the carcasses placed at 37° C for thirty minutes. After this incubation the groin and axillary masses about the encapsulated casein were fixed in Zenker's fluid, embedded in paraffin and sectioned. The inflammatory reaction and the character of the granulation tissue varied with the time following the casein injection, but in all animals there were numerous newly formed vessels in the sections. Although the lungs, liver and spleen were a slate color, the capillaries about the encapsulated casein contained comparatively little ink. Ink within the endothelial cells was so scant that its presence there was questionable. Ink-containing phagocytes within the vessels were readily demonstrable. Later a rabbit, while being injected intravenously with India ink, died suddenly and was incubated for thirty minutes. As was the case with the rats, the endothelium was practically devoid of carbon.

Experiment B: Each of two young rabbits, weighing 675 gm. and 750 gm., received on June 1 six subcutaneous injections of casein. On June 25 each received an intravenous injection of 0.5 cc. India ink. On July 6 at 10 A.M. each received 1 cc. of India ink intravenously. Two hours later the animals were chloroformed and the lumps of encapsulated casein at once fixed in Zenker's fluid.

Although the liver sinusoids contain huge masses of agglutinated ink particles there is comparatively little ink in the casein nodules. The description and illustrations are of the two rabbits with double ink injection. Multiple microscopic nodules have formed about

small masses of casein. At the centers are casein particles encircled by a zone of polymorphonuclear leukocytes. The next zone consists of granulation tissue with a varying proportion of large mononuclear phagocytes, giant cells, lymphocytes, polymorphonuclears, new blood vessels, and connective tissue. About one per cent of the large mononuclear phagocytes contain carbon and more than one-half of the giant cells are marked with carbon. This carbon, and also the abundant carbon in the periportal connective tissue in the liver, is evidently from the first ink injection. Endothelial cells with ink particles are most abundant in the periphery of the granulation tissue abutting on muscle, adipose tissue, or loose connective tissue. Practically no free carbon is present in the lumina of the vessels but intravascular mononuclear phagocytes marked with ink occur. The capillaries within the dense leukocytic zone toward the casein contain carbon but in the sections they are collapsed and devoid of the red blood corpuscles which are so helpful in identification. It is therefore at the periphery of the casein nodules that discrete definite carbon particles are best seen within the cytoplasm of the endothelium of capillaries. Usually the vessels that show best are in longitudinal section and are sufficiently dilated to be cut through the walls on each side (Figs. 2 and 3). When the vessel wall in longitudinal section is not cut through it is often difficult to determine whether the carbon is intracellular or within the vessel lumen. To find a capillary cut across with carbon in the wall requires considerable search (Fig. 4). Taking into account the relatively small quantity of ink present in the nodules, it is obvious that it is only in an occasional capillary that the endothelial cells make contact with the carbon so as to permit phagocytosis.

Discussion

Since these experiments show that vascular endothelium of granulation tissue may phagocyte carbon, just as the stimulated endothelium of various organs was found to do in earlier experiments, the blood vascular endothelium must be considered as a possible source of mononuclear phagocytes. Mallory [7] was the first to recognize the endothelial origin of the mononuclear phagocyte of the blood and tissues. In a discussion of the histology of typhoid fever lesions that author [8] stated that not only the vascular endothelium but also

the reticulo-endothelium of the lymphoid tissue gave origin to endothelial leukocytes. By marking the endothelium with carbon experimentally, I [6] reached the conclusion that the mononuclear phagocytes were of endothelial origin. The deduction was based chiefly on the structural resemblance between carbon-containing endothelial cells and the free phagocytes with carbon. The reticulo-endothelium of lymphoid tissue, as well as the blood vascular endothelium, was found to phagocyte the carbon and at this time was stated to be a source of mononuclear phagocytes. There were not sufficient data to determine which of the two types of endothelial cells was the major source of these phagocytes. Sabin, Doan and Cunningham [5] summarize as follows: "Thus we consider that the weight of evidence points to two separate strains of phagocytic cells of the connective tissue: clasmatocytes, which are of endothelial origin and come into the blood stream only occasionally and abnormally, and monocytes, which are a constant type of blood-cell, arising largely in the spleen, but also a specific cell of the diffuse connective tissues, where they both arise and function." My own work,[9, 10, 11, 12, 13, 14, 15] convinces me that the conclusion reached by Sabin and her associates in regard to the clasmatocyte (hemendotheliocyte) is correct. By experimental procedure this cell may be made to enter the blood stream. Doubtless it usually arises from active endothelium such as the Kupffer cell, but capillary endothelium elsewhere (heart, granulation tissue) has the same potentiality. Also I agree with these investigators that there is a second type of phagocyte, the monocyte; but my experiments tend to show that this cell is derived from the reticular portion of lymphoid tissue wherever found. This lymphendotheliocyte is practically the sole mononuclear phagocyte of normal peripheral blood. It is also the usual mononuclear phagocyte of a variety of lesions. No conclusive work exists to show whether there is a relationship between the reticular and lymphocytic cells of lymphoid tissue. Once differentiated, I have found no evidence that the lymphocyte may enlarge to become a reticular phagocyte.

Conclusions

1. Carbon is present in the vessel walls of granulation tissue after India ink has been injected intravenously and also to some extent at the site of subcutaneous ink injections.

2. The presence of this particulate matter within the cytoplasm of the endothelial cells can be explained only as a process of active phagocytosis.

3. The phagocytic activity of these endothelial cells is identical with that of the Kupffer cells of the liver which have a comparable amount of cytoplasm.

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DESCRIPTION OF PLATE

PLATE 136

FIG. 1. Carbon in endothelial cells of a dilated vessel at site of subcutaneous ink injection. Section is tangential and vessel contained red corpuscles beyond portion in illustration.

FIG. 2. Capillary with phagocyted carbon from periphery of casein nodule of rabbit (Experiment B).

FIG. 3. Tangential section of vessel near periphery of a nodule (Experiment B). Heavy carbon masses in endothelial cells.

FIG. 4. Transverse section of capillary, well within a casein nodule (Experiment B).

NOTE: The structures were outlined with camera lucida. The drawings were made by Miss M. E. Bakehouse of the Department of Anatomy.

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McJunkin

Phagocytic Activity of Vascular Endothelium

PRIMARY CARCINOMA OF THE LIVER: TWO CASES IN CATTLE *

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The spontaneous formation of tumor in the liver is not common. The organ suffers more often as a repository for metastasis from primary tumor elsewhere in the body.

According to Ewing, primary carcinomas of the liver constitute between 0.5 and 1.3 per cent of all epitheliomas in man. Authentic data concerning the incidence in the lower animals are very meager; Sticker's [1] figures are perhaps the most comprehensive. He reported the liver to be the site of primary origin in thirty-five of a total of 1206 primary carcinomas of the common domesticated animals. These thirty-five cases were distributed among the various species as follows: three in horses, four in oxen, five in sheep, twenty in dogs, two in cats, and one in a hog. In sixty-three cases of carcinoma and adenocarcinoma of the lower animals reviewed by Fadyean,[2] six cases of primary carcinoma of the liver are listed. These include three in dogs, two in sheep, and one in a cow. Hodgson [3] described one case of primary carcinoma of a sheep's liver. The remainder of the abdominal viscera were not examined and Hodgson was in doubt as to whether the tumor was primary in the liver. However, in view of the extreme rarity of carcinomas in the gastro-intestinal tract of sheep, and the fact that most internal tumors of the sheep have been carcinomas of the liver, I believe that Hodgson's case was primary in this organ. Murray [4] listed seven cases of malignant adenoma of the liver in a total of twenty-four malignant tumors in cattle. Primary carcinoma of the liver was not observed in twelve cases of malignant tumors in horses, and in eleven in cats. Murray did, however, find a "columnar cell carcinoma" in the liver of an old dog in a total of forty-nine malignant cases in dogs. In a case described by Ross [5] a primary carcinoma of the liver, invasive in nature, was found in a dachshund. From Ross' description one is

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led to believe that his case represented a true carcinoma of the liver. Cohrs [6] reported several cases of multiple neoplasm in dogs in which carcinoma of the liver was present and one case in a cat, associated with a cystadenoma of the gall ducts. Fox [7] found an adenoma simplex of the liver of a woodchuck. Hutyra and Marek [8] stated that primary carcinoma of the liver is rare in all the domestic animals but is most common in the dog. Kitt [9] noted this in old dogs. Roussy and Wolf [10] stated that the liver of cattle is frequently the site of adenomas, epitheliomas and sarcomas, and that they are usually associated with the presence of parasites (nematodes). Roussy and Wolf also mentioned that the parasitic livers of sheep not infrequently contain tumors similar to those found in cattle.

Siedamgrotzky [11] reported the case of a primary carcinoma of the liver in a hen with metastasis to the intestines. Joest and Ernesti [12] described two cases of primary carcinoma of the liver in a series of twenty carcinomas of birds; in one case the carcinoma was widely disseminated throughout the organ. Metastasis had not occurred. Pentimalli [13] did not observe primary carcinoma of the liver in a series of eighteen chicken tumors. In my study of twenty-seven neoplasms in chickens I did not find evidence of primary carcinoma of the liver. I believe it is one of the rare tumors in chickens.

By far the most comprehensive description of primary carcinoma of the liver in cattle is that by Trotter.[14, 15] He had the unusual opportunity to study 119 cases which were found in a total of 39,704 slaughtered cattle during one year at a Glasgow abattoir. Later he reported another case. Of the 120 cases all but two were in animals from the north or northwest of Ireland. The condition was more common in the older animals, "frequently associated with adenoma, chronic venous congestion, cirrhosis, distomatosis, cavernous angioma or, but most rarely, tuberculosis."

In one of Trotter's cases the affected liver weighed 36.3 kg. (80 pounds). Trotter gives a splendid picture of the metastatic possibilities of primary carcinoma of the liver and mentions that on several occasions emboli of tumor cells were found firmly attached within the lumen of one of the smaller branches of the portal vein. He observed that the thrombi were found more often in the lumen of the branches of the portal vein than in those of the hepatic artery or vein, and believed that the growing tumor cells cause so much pressure on the walls of the vessels as to invaginate the walls.

The tumor continues to expand and eventually the wall of the vessel ruptures, permitting the thrombus of tumor cells to project into the lumen of the vessel. Such a situation favors the dissemination of those tumor cells which become loosened from the parent mass, and as a consequence distant metastasis is possible. Trotter found instances of secondary foci in the lungs and in one case he found metastasis to the cortex of the kidney. Although the hepatic lymph nodes were often affected, metastasis occurred more frequently by way of the blood stream. Trotter also noted a "villous-like growth" in the lumen of the gall-bladder which had broken through the walls from its primary focus in the liver. In only one case was the peritoneum involved and in this case both the visceral and parietal surfaces were studded with secondary nodules of varying sizes. In this instance the hepatic, renal, and right internal iliac lymph nodes were also involved. Trotter observed a few cases in sheep.

In my series of eighty cases of neoplasms in cattle there were two of primary carcinoma of the liver. These are reported herewith.

Report of Cases

Case I. Material was received from a veterinarian in the federal meat inspection service, Denver, Colorado. The animal was a four-year-old Herford cow in apparent health and was slaughtered for food. Examination of the carcass, however, showed that the parietal surface of the liver presented several small nodular grayish tumors measuring from 0.5 to 2 cm. in diameter, which were grouped around a larger central mass measuring about 7 cm. in diameter. A few areas of necrosis and calcification were also observed. Because of these unusual lesions the veterinary inspector made a thorough examination of the head, lungs, kidneys, spleen, adrenals, gall-bladder, bones, lymph nodes, and intestines, but evidence of other tumors was not found.

Histologic Examination: This showed the normal structure of the liver to be interrupted by irregular collections of closely packed cells which stained strongly acidophilic. The larger areas of tumor cells were definitely separated from the tissue of the liver by broad bands of dense fibrous connective tissue. The smaller collections of cells did not have such barriers between them and the liver and seemed to be undergoing extension by infiltration into the remaining tissue of the liver (Fig. 1).

The parenchyma of the tumor consisted of large polyhedral cells resembling in many respects hepatic cells but lacking an orderly arrangement. The cytoplasm was minutely granular but a definite

cell wall could not be demonstrated. The nuclei were also large and in practically every instance a prominent nucleolus could be seen. Many of the nuclei appeared vesiculated and in the more immature forms they were strikingly hyperchromatic. Mitotic figures, while not abundant, were nevertheless frequently seen (Fig. 2).

Blood capillaries were present among the tumor cells, as were spaces suggestive of bile capillaries, but bile ducts could not be demonstrated. Sections stained by Best's carmine method revealed the presence of glycogen in the tumor cells.

The hepatic cells adjacent to the tumor were compressed and atrophic and were arranged in slender columns running parallel to the fibrous connective tissue which separated the neoplastic elements from those of the liver. The capsule of the organ was irregularly thickened and some of the subcapsular areas showed marked congestion of the blood capillaries. Extensive lymphocytic infiltration was a conspicuous feature of most of the fields (Fig. 1). The lymphocytes were distributed among the various collections of tumor cells and seemed to be most abundant in areas in which a reticular stroma was present. Cirrhosis was not observed. A diagnosis was made of primary carcinoma of the liver.

CASE II. A veterinarian in the federal meat inspection service also supplied the material for this case. The animal was an old-grade cow in very poor condition and the carcass was condemned on account of emaciation. At necropsy a large, flattened mass was found extending slightly above the parietal surface of the liver. The mass measured 20 by 12 cm.; it was firm, and of a dirty flesh color. On cross-section the mass was found to be rather sharply separated from the surrounding scant amount of liver tissue, which was greatly compressed by the expanding tumor (Fig. 3). There were many diffuse brownish areas throughout the mass and a few cystic cavities were apparent. Metastatic deposits were not found on careful examination of the carcass.

Histologic Examination: The substance of the liver in this case could barely be detected, the tissue consisting mostly of wide areas of large, strongly acidophilic cells not unlike those described in Case I. The type cell was polyhedral in contour with a finely granular cytoplasm and a large nucleus containing a prominent nucleolus. Most of the nuclei contained many chromatin granules and a few were vesiculated (Fig. 4). A few cells undergoing mitotic division were seen. The cells were without apparent arrangement, being disposed in a haphazard manner with an occasional suggestion of small

groups or clumps. Blood capillaries were generously distributed among the tumor cells, but bile ducts were not demonstrable.

The tumor was cut at irregular intervals by wide strands of fibrous tissue and in the few areas in which hepatic tissue remained an occasional large pink homogeneous cyst was found. The few remaining hepatic cells were atrophic and disarranged from the effects of the pressure exerted by the encroaching neoplasm. There was considerable extravasation of blood in some areas, which explained the diffuse brownish-colored portions observed in the cross-section of the gross tumor. The round-cell infiltration which was so noticeable in Case I was not observed. In a few areas the tumor cells had undergone distinct hyaline change. Cirrhosis was not observed. Glycogen was demonstrated by Best's carmine stain. A diagnosis of primary carcinoma of the liver was made.

Comment

Three types of primary epithelioma of the liver may occur. Only two of these have been described in cattle. In one the neoplastic cells have their progenitors in the biliary epithelioma of the bile ducts with the resultant growth adenomatous in character. Another arises from the parenchymatous cells of the organ. Both of the tumors described here were of the latter variety, and the term "hepatoma" which has been used by certain observers in designating primary carcinoma of the liver could be used at least clinically in designating this type.

The third type of epithelial tumor which may arise from the liver, according to Mallory,[16] is from misplaced cells from the suprarenal cortex. I have not found such a tumor reported in any of the lower animals.

The multiple nodules observed in Case I suggest two possibilities: either that the disease had a multicentric origin, or that the smaller nodules arose secondarily from the primary focus. The clinical, necropsy, and histologic data favor the latter explanation.

The designation of both of these tumors as carcinomas is justified: in Case I by the presence of the aggressive infiltrative manner of growth and many immature cells, many of which revealed mitosis. In Case II the designation of carcinoma is more difficult to defend. Secondary foci were not demonstrated and much of the tumor was

definitely encapsulated by a formidable connective tissue barrier. The mitotic figures, however, and the large numbers of immature cells, with the abundant possibilities for the tumor cells to be carried by the blood to distant situations, make a diagnosis of primary carcinoma of the liver tenable, if not absolutely conclusive.

In man the greater percentage of cases of primary carcinoma of the liver are associated with cirrhosis. Ewing [17] stated that cirrhosis is the chief predisposing factor and that it occurs in about 85 per cent of cases. McIndoe and Counseller [18] also emphasize the probable relationship between cirrhosis and hepatoma and say that it is the main predisposing factor in the disease.

The rôle of cirrhosis in the production of primary hepatic carcinoma is explained on the basis of a regenerative overgrowth of injured or degenerated cells with a resultant loss of restraint and the assuming of autonomous tendencies.

Contrary to the foregoing observations, cirrhosis was not demonstrated in either of the cases forming the basis of this report. Accurate data concerning the incidence of cirrhosis in the various domesticated species are not available, although Hutyra and Marek quote Tschauner as having found three cases of hepatic cirrhosis in 5700 hogs. Although cirrhosis does occur sporadically in the lower animals it does not constitute one of the major pathologic alterations due, perhaps, to the absence in the lower animals of many of the contributory factors frequently associated with the etiology of portal cirrhosis in man. One cannot avoid the inference that primary carcinoma of the liver would be more frequently seen in animals if portal cirrhosis occupied the same relative position in the diseases of animals that it does in diseases of man.

The possible relation of parasitism to primary carcinoma of the liver is suggested from the observations of Roussy and Wolf who reported the presence of nematodes in the liver of cattle affected with primary epithelioma. The liver of sheep affected with similar tumors often harbors parasites, according to these writers.

In these cases it seems reasonable to assume that the toxic products of the parasite and the mechanical irritation of their presence could well incite a degenerative injury of the hepatic cells that would be followed by a regenerative attempt at restoration with resultant cirrhosis and perhaps a neoplastic change in the cells of the parenchyma.

The conclusion one must draw from an examination of the cases reported in the literature is that ordinarily primary carcinoma of the liver is not a tumor of vicious malignancy. Occasionally these tumors cause widespread distribution, but as a rule their activities are limited to the organ responsible for their histogenesis. Occurring as they do in older animals, it is not surprising that serious hepatic involvement should contribute to the general debility so often seen in old cows with subsequent emaciation or perhaps cachexia.

Summary

From a review of the literature one must conclude that primary carcinoma of the liver is not one of the common tumors of the lower animals. Of the domesticated species the dog seems to be the most often affected. The tumors have also been reported in the following species: horse, cattle, sheep, cat, hog, woodchuck and chicken. In one series reported by Trotter one hundred and nineteen cases were found in 39,704 necropsies on cattle.

The true primary carcinoma of the liver arises from the parenchymatous hepatic cell, and while the tumor is occasionally extremely malignant it usually exerts its major influence on the hepatic substance in which it arises, metastasis being the exception rather than the rule.

Two original cases of primary carcinoma of the liver are reported, both occurring in cattle. Metastasis was not observed in either case.

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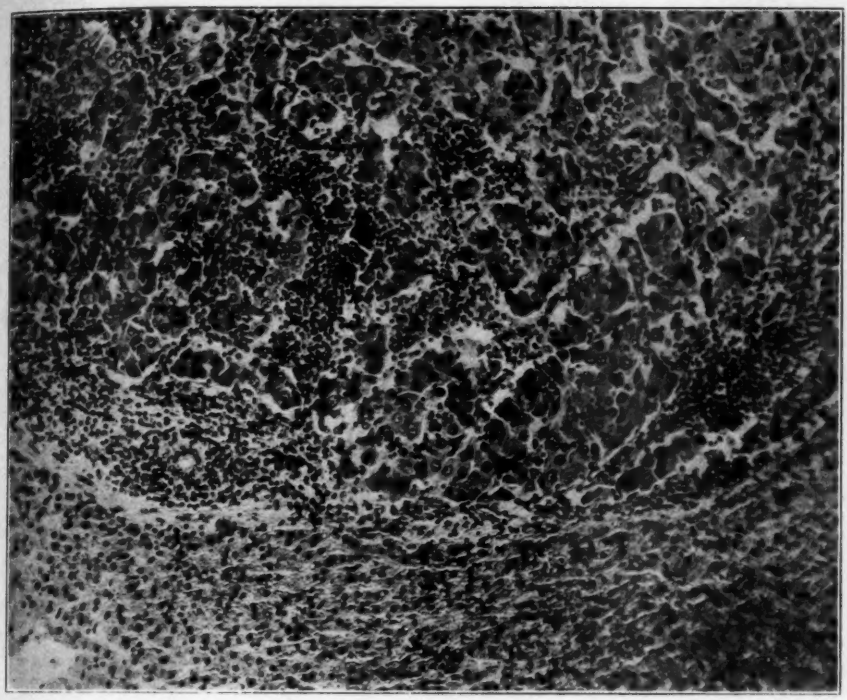
DESCRIPTION OF PLATES

PLATE 137

FIG. 1. CASE I. Primary carcinoma of the liver. Advancing cells of the tumor pushing outward into the tissue which is much compressed and atrophic. The extreme lymphocytic infiltration is apparent. × 150.

FIG. 2. CASE I. Primary carcinoma of the liver. One cell in mitosis. × 1350.



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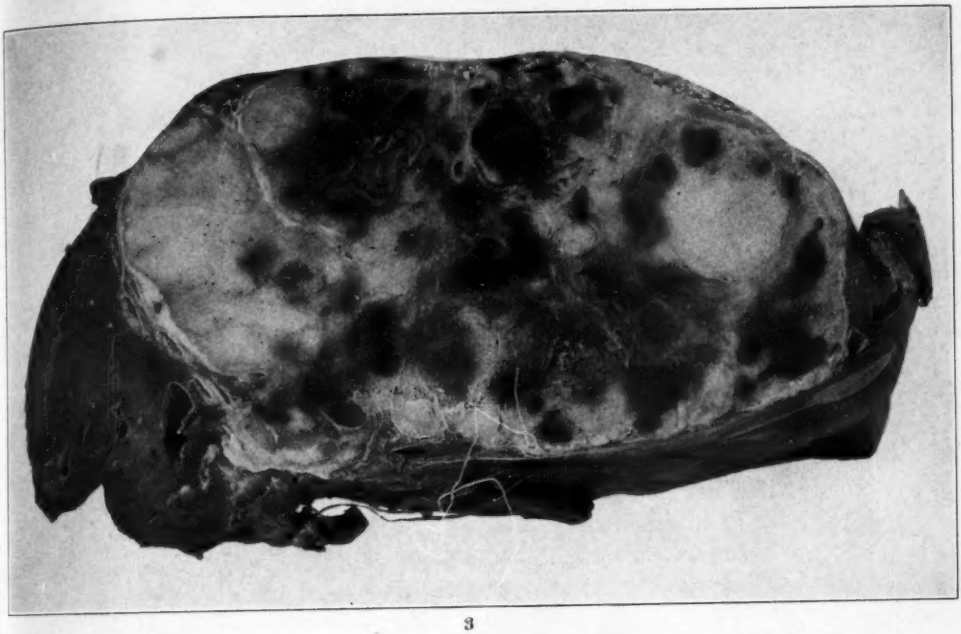
2

Feldman Primary Carcinoma of Liver

PLATE 138

FIG. 3. CASE II. Primary carcinoma of the liver. A cross-sectional view of the gross specimen. The small amount of liver tissue and the areas of hemorrhage are shown.

FIG. 4. Primary carcinoma of the liver showing the compactness of the tumor cells and the absence of a regular arrangement. × 550.



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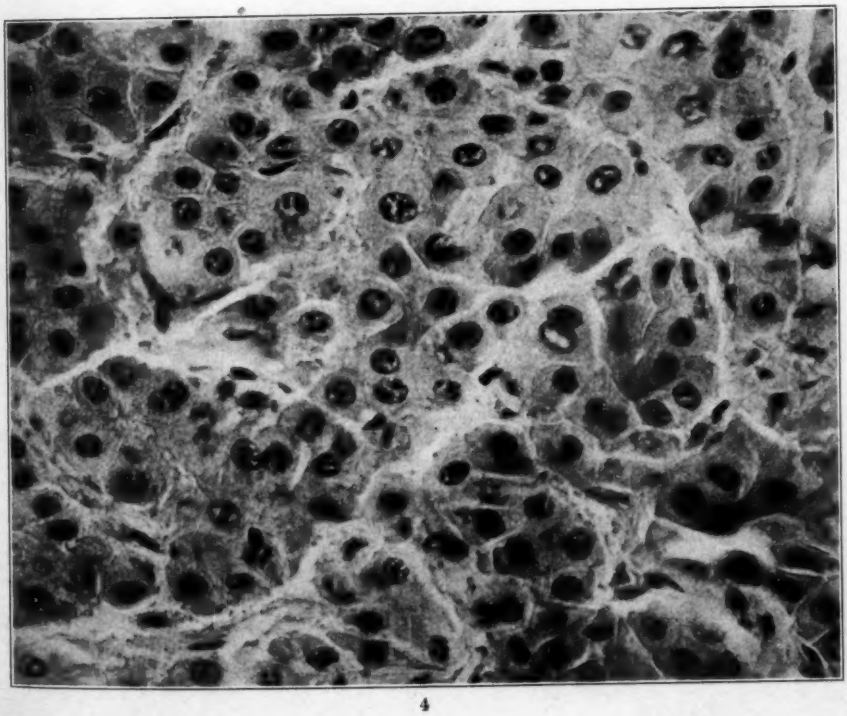

4

Feldman

Primary Carcinoma of Liver

THE EFFECT OF FEEDING POTASSIUM IODIDE ON THE PROLIFERATIVE ACTIVITY OF THE THYROID GLAND IN GUINEA PIGS*

JACOB RABINOVITCH

(From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.)

According to Marine and Lenhart,[1] the administration of potassium iodide prevents compensatory hypertrophy of the thyroid gland in dogs, in which parts of the thyroid gland have been removed. Associated with the diminished activity of the gland they found a distinct increase in the amount of colloid in the acini. These authors also pointed out that if iodine is fed to dogs the amount of this substance taken up by the thyroid depends upon the quantity of iodine administered, the degree of hyperplasia and the extent of the epithelial surface; the greater the degree of hyperplasia the more iodine is taken up.

In the course of his studies on compensatory hypertrophy, Loeb [2, 3, 4] found that contrary to the conclusions of the previous investigators, administration of potassium iodide not only does not prevent compensatory hypertrophy in guinea pigs, after removal of part of the thyroid gland, but that it even increases on the average the intensity of compensatory hypertrophy. In a subsequent article Marine [5] reiterated his statement that potassium iodide prevents compensatory hypertrophy and he attributed the contrary results of Loeb to the fact that the latter had removed a very large part of the thyroid gland in the animals in which he wished to produce compensatory hypertrophy. However, Gray, Haven and Loeb,[6] and Gray and Loeb [7] could show that in guinea pigs in which the thyroid gland had been left intact, the administration of potassium iodide, instead of decreasing the activity of the thyroid gland, caused a very marked increase of the mitotic activity of the gland during the first three or four weeks following the feeding with potassium iodide. But if the potassium iodide was continued for a longer period of time, an increase in the quantity of colloid, usually associated with

* Received for publication July 2, 1928.

an increase of fluidity of this substance, took place, and, as a result of this process, pressure was exerted on the acinus cells, which was injurious to the latter and brought the proliferation to a standstill.

In continuation of the work of the latter authors, we attempted to determine in a quantitative manner the variation in mitotic activity at various periods during the administration of potassium iodide, and furthermore, we wished to determine whether there existed a relation between the quantity of iodide administered and the intensity of the proliferative process produced.

The experiments were carried out on four sets of guinea pigs, weighing on the average between 350 and 450 gm.; some of these were fed with varying quantities of potassium iodide for different periods of time, while others were kept as controls. The first set of animals received a daily dose of 0.01 gm., the second set 0.05 gm., and the third set 0.1 gm. of potassium iodide. The fourth set were kept as controls and did not receive any potassium iodide. The iodide was administered in the form of pills fed by mouth, great care being taken that all of the substance was swallowed. In different groups of animals the feeding was continued for periods of 10, 15, 18, 20 and 30 days respectively, and at the end of each period the animal was killed with chloroform, and both lobes of the thyroid, excluding the isthmus, were immediately removed and at once fixed in Zenker's solution. The glands were cut in complete serial sections and stained with hematoxylin and eosin. The average number of sections obtained for each gland varied between 450 and 500; mitoses were counted in every tenth section in a very exact manner; the number of mitoses thus obtained was multiplied by ten in order to find the total number of mitoses in the thyroid gland. Changes in body weight of the animal during the period of the experiment were also recorded, because, as will be shown in a separate paper, the experiments of Loeb as well as my own show a definite relation between the loss and gain in body weight and the mitotic activity in the thyroid gland. We shall first discuss the changes observed in the number of mitoses in the thyroid epithelium under the influence of potassium iodide feeding; this will be followed by a description of the changes which take place in the thyroid gland under similar conditions.

I. Counts of Mitoses

(*A*) *Control Animals:* The number of mitoses found in the thyroid gland of our control animals varied in the majority of cases between 135 and 250. (See Table I.)

Table I

Mitoses, Control Series

	10 days	15 days	18 days	20 days	30 days
	180	135	440	60	380
	0	190	196	140	120
	200	156	0	40	235
Averages of mitoses	127	160	212	80	245

Of fifteen control animals, in eight the number of mitoses ranged between 135 and 250, in two they were 380 and 440 respectively, and in five they were below 135. The total average of mitoses was 165, with the highest number reaching to 440 and the lowest being zero.

(*B*) *Counts of Mitoses in Animals Fed with KI for a Period of 10 Days:* Animals that were fed with potassium iodide for ten days showed a relatively slight increase in the number of mitoses as compared with the controls. (See Table II.) There was found furthermore a slight increase in the number of mitoses in passing from animals which were fed with smaller doses of potassium iodide to the animals which were fed with the larger doses. The averages were 280 in the animals receiving 0.01 gm., 370 in those receiving 0.05 gm. and 420 in those receiving 0.1 gm. of potassium iodide.

Table II

KI Feeding 10 days

	0.01 gm.	0.05 gm.	0.1 gm.
	200	320	420
	360	420	400
	240	400	440
	320	340	410
	...	...	430
Averages of mitoses	280	370	420

(*C*) *Counts of Mitoses in Animals Fed with KI for a Period of 15 and 16 Days:* In this group of animals a remarkable increase in mitoses has taken place over the number found in the controls as

well as in the animals fed with potassium iodide for ten days; moreover, the increase is greater, the larger the quantity of iodide administered. (See Table III.)

TABLE III

KI Feeding 15 days

	0.01 gm.	0.05 gm.	0.1 gm.
	2100	3210	6884
	1340	3790	3442
	1910	....	3440
	1530	....	6890
Averages of mitoses	1720	3500	5164

We may therefore conclude that the stimulating effect of potassium iodide on the proliferative activity of the thyroid gland becomes very effective after almost two weeks of iodide feeding and that the effect is greater the larger the quantity of iodide given. The averages found when potassium iodide had been administered for a period of fifteen days were 1720 mitoses, 3500 mitoses and 5164 mitoses in animals fed with 0.01, 0.05 and 0.1 gm. respectively. The highest number of mitoses in this group was 6890 and the lowest number was 1340, the latter figure being found in the animal fed with the smallest quantity of potassium iodide. The number of mitoses in animals fed with 0.1 gm. of potassium iodide was therefore 31 times as great as in the control animals, while it was 21 times greater in animals fed with 0.05 gm. and 10 times greater in animals fed with 0.01 gm. of potassium iodide. The number of mitoses in the animals fed with 0.01, 0.05 and 0.1 gm. of potassium iodide show therefore an approximate ratio of 1:2:3.

Experiments, extending over a period of sixteen days, showed the same results. (See Table IV.)

TABLE IV

KI Feeding 16 days

	0.01 gm.	0.1 gm.
	1800	3361
	1560	5280
Averages of mitoses	1680	4320

Two of four guinea pigs, which were of approximately the same weight, fed with 0.01 gm. potassium iodide showed an average of

1680 mitoses, while two guinea pigs fed with 0.1 gm. showed approximately two and one-half times as much, namely, an average of 4320 mitoses.

(*D*) *Counts of Mitoses in Animals Fed with KI for 18 Days:* The feeding of potassium iodide to guinea pigs during a period of eighteen days is also characterized by an increased proliferative activity of the thyroid epithelium, which in the case of the smallest dose is somewhat more intense than that observed in the animals fed with potassium iodide during a period of fifteen days. (See Table V.) The relation in this experiment between the number of mitoses found after feeding with 0.01, 0.05 and 0.1 gm. potassium iodide varies according to the ratio of 1:1.2:1.8 respectively.

TABLE V

KI Feeding 18 days

	0.01 gm.	0.05 gm.	0.1 gm.
	1520	3480	5300
	4960	3170	5340
	2036	3790	4960
	3240	....	5680
Averages of mitoses	2939	3480	5320

(*E*) *Counts of Mitoses in Animals Fed with KI for a Period of 20 Days:* In this group we find essentially the same conditions as those described in periods of fifteen and eighteen days. However, in the case of animals fed with 0.05 and 0.1 gm., there was observed a further increase in the number of mitoses. (See Table VI.) In this period the highest counts in mitoses were observed in animals fed with 0.05 and 0.1 gm. of potassium iodide. They exceeded the average counts in any of the previous periods.

TABLE VI

KI Feeding 20 days

	0.01 gm.	0.05 gm.	0.1 gm.
	2226	4190	8000
	2250	4216	....
	2160	4218	....
Averages of mitoses	2212	4208	8000

But we must consider the fact that only one animal was counted which had received 0.1 gm. of potassium iodide for twenty days and

in which the number of mitoses was 8000. The proportion in counts in the animals fed with different quantities of iodide varied according to the ratio of 1:1.9:3.6. We find then during the third week a most remarkable increase in the number of mitoses throughout the various doses of the iodide feeding, which is greater in cases where larger quantities are fed.

(*F*) *Counts of Mitoses in Animals Fed with KI for a Period of 30 Days:* In experiments in which iodide feeding was continued for thirty days, the marked proliferation of the thyroid epithelium was lacking. (See Table VII.)

TABLE VII

KI Feeding 30 days

	0.01 gm.	0.05 gm.	0.1 gm.
Mitoses	40	120	600

The average number of mitoses found here was approximately within the range noted in our control animals not fed with potassium iodide. There was perhaps a very moderate increase in the guinea pigs which had received 0.1 gm. of iodide. We may therefore conclude that secondary factors cause cessation of the stimulating effect which is produced with potassium iodide when administered for a period of over two to three weeks.

TABLE VIII

Summary of Results of KI Feeding

Time	Controls	0.01 gm.	0.05 gm.	0.1 gm.
10 days	127*	280	370	420
15 days	160*	1720*	3500*	5164*
16 days	....	1680*	....	4320*
18 days	212*	2939*	3480*	5320*
20 days	80*	2212*	4208*	8000*
30 days	245*	40	120	600
*Averages of mitoses	165 (15)	2138 (13)	3729 (8)	5701 (11)

The previous experiments were carried out during the cooler season of the year. An earlier series of experiments, of a similar character to those reported above, were carried out during the summer months. Unfortunately, in the summer series, the glands were not sectioned completely, and consequently the number of mitoses in the entire gland could not be determined accurately. The study

of the sections which were available showed, however, that they agreed with our conclusions as to the stimulating effect of potassium iodide feeding on the thyroid gland. In these experiments also, the greatest numbers of mitoses were found in animals fed with potas-

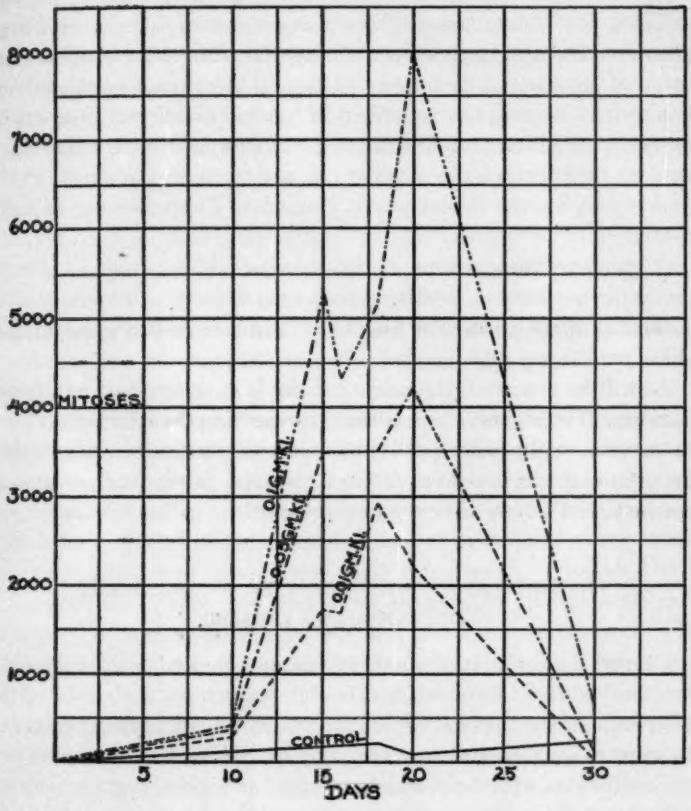

Chart I

sium iodide for about two to three weeks, and furthermore, those guinea pigs which received the larger quantity of iodide showed a greater proliferative activity.

Considering all these experiments we may conclude that potassium iodide stimulates proliferative activity in the thyroid in an extraordinary manner; that the stimulation is very definite two to three

weeks after the feeding of the iodide and that the larger doses cause a greater stimulating effect than the smaller doses within the range of quantities administered by us. Thirty days after the beginning of the experiments secondary factors have begun to become active which counteract the proliferation of the epithelial cells.

Table VIII summarizes the results obtained by us, omitting however the experiments done during the summer, the latter not being of an exact, quantitative nature. The average of mitoses in the control animals is 165, while the number of mitoses in animals fed with the different quantities of potassium iodide for a period of two to three weeks are 2138, 3729 and 5701 respectively. The figures in parentheses indicate the number of experiments in each case.

A graphic representation of the changes taking place in animals under the influence of feeding various quantities of potassium iodide as well as of the conditions found in control animals is given in the chart.

As will be observed, the control curve is very low and relatively uniform. The highest curve is that representing the effect of 0.1 gm. potassium iodide feeding; the maximum of the various curves lies between eighteen and twenty days, the base is reached or almost reached thirty days after the beginning of the iodide feeding.

II. Changes in the Character of the Acini, Epithelium and Colloid

Control Animals: In the control animals we find small or medium-sized alveoli, lined with cuboidal epithelium and filled with solid colloid; but certain variations occur from this typical picture. In some cases the acini may be larger or smaller than the average, the epithelium may be somewhat flatter or higher, and the colloid softer and filling the alveoli very incompletely. Occasionally also a moderate number of phagocytes may be found in the colloid of various acini. Furthermore, collections of lymphocytes may be noted, in some instances, although these are not frequent occurrences.

Animals Fed with Potassium Iodide: During the first ten days of potassium iodide feeding, no marked changes in the structure of the thyroid gland are observed. In the animals fed for a period of fifteen

to twenty days with potassium iodide, however, some changes may be observed, although they are on the whole slight. The acini, as a rule, are of medium size or perhaps a little larger and regular in outline; the cells are usually somewhat increased in size particularly in cases where many mitoses are found. However, this does not hold good in every instance; at times we find areas where the epithelium is relatively low or flattened out. There is noticeable a slight tendency on the part of the colloid to soften and to retract from the margins of the epithelial lining of the acini. The most striking feature observed, however, in this group of animals, is the very pronounced increase in the number of phagocytes, which we find in the colloid of the acini. This increase in phagocytosis under the influence of potassium iodide has also been noted by Gray and Loeb. At the same time some softening and solution processes take place in the colloid which may go so far that the latter may disappear from the lumen of some of the acini. The phagocytic activity is probably due to changes which the feeding with potassium iodide produces in the thyroid gland.

The histological appearance of the thyroid, obtained from animals that were fed with potassium iodide for thirty days, shows more pronounced changes than those of earlier periods. These changes concern the acini and epithelium as well as the colloid. After thirty days the acini are large and sometimes irregular in outline, owing to the occasional fusion of two or three neighboring acini. Associated with this increase in size and occasional irregularity of the acini is a lowering or even complete flattening of the lining epithelium. The colloid becomes very soft or it may be entirely liquefied. In accordance with the view expressed by Gray and Loeb [6,7] we are inclined to consider the changes in the colloid as primary and the changes in the character of acini and epithelium as the result of mechanical pressure exerted by the increased contents of the acinus, on the wall of the latter. The phagocytic activity which was increased in the previous period, is less marked in the acini thirty days after the beginning of the KI administration.

Maintenance of Body Weight: In the experiments of Loeb on the hypertrophy of the thyroid gland, there was noticed a relation between the degree of hypertrophy and the changes in the weight of the animal which took place during the course of the experiment. A gain in weight during this period was a condition favorable to

hypertrophy, while a loss in weight had the opposite effect.[8] The same relation was observed by us in our present experiments; the thyroid epithelium showed more active proliferation in the former than in the latter case. We shall discuss this relationship in a subsequent paper.

Discussion and Conclusions

We consider the results which we obtained in our experiments as very significant. They show quite conclusively that potassium iodide, when given to normal guinea pigs in the manner we described above, induces a pronounced proliferation of the acinus cells in the thyroid gland, as evidenced by the marked increase in the number of mitoses. The increase in cell division, resulting from the iodide feeding, is most intense between the fifteenth and twentieth day of the administration of this substance. It is far less pronounced or is absent during the first ten days of the iodide feeding, and it decreases again very markedly when treatment is prolonged to thirty days. At this latter period the proliferation is even less marked than at the tenth day. It is furthermore noted that, within the range of our experiments, the greater the dose of the iodide administered to the animals, the more intense is the degree of mitotic cell division. This quantitative relationship between the dose of potassium iodide given and the intensity of cell proliferation is brought out very clearly in our tables.

Structural changes in the thyroid as far as they relate to the character of the colloid, of the acini and the lining epithelium, are lacking in the first ten days of the iodide feeding; between the fifteenth and twentieth day, the changes observed are very slight and consist in a somewhat softer colloid and perhaps a slightly higher epithelium, while the acini remain of about the same size. During this stage we notice, however, the appearance of a very large number of phagocytes in the colloid. After thirty days of iodide feeding, there occur more appreciable changes; the acini become large, irregular and often fuse together, the colloid is soft and edematous and the cells are low and flattened out. The phagocytes now tend to diminish or disappear entirely.

Summary

1. A quantitative estimation of the proliferative activity of the thyroid gland has been carried out, by counting the number of mitoses present in the normal gland as well as in the glands of guinea pigs fed with various quantities of potassium iodide during different periods of time.

2. The number of mitoses found in thyroids obtained from animals fed with potassium iodide within the first three weeks after the beginning of the feeding is by far greater than that observed in the controls. The increase in the number of mitoses is only slight during the first ten days. It becomes very pronounced between the fifteenth and twentieth day of the iodide feeding, and lessens again when feeding is continued for thirty days.

3. Within the range of quantities of the substance used by us, larger doses of potassium iodide call forth greater proliferative activities of the thyroid epithelium than smaller doses.

4. At the same time the colloid becomes slightly softer and the phagocytic activity within the colloid increases after potassium iodide has been fed for fifteen to twenty days; the epithelium, however, changes very little in height. At the end of the thirtieth day, the acini enlarge and become irregular, the colloid becomes watery and the epithelium flattens out; the number of phagocytes also diminish. We attribute the flattening of the acinus epithelium, which is observed in the fifth week, to the pressure exerted on the epithelium by the increase in volume in the colloid resulting from the absorption of water at this period.

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SCIENTIFIC PROCEEDINGS OF THE TWENTY-EIGHTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

WASHINGTON, D. C.

MAY 1, 2 AND 3, 1928.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

SEROLOGICAL DISCORD IN LATENT AND TREATED SYPHILIS. Adele E. Sheplar and Morris A. Lyons (by invitation) and Ward J. MacNeal, New York City.

Abstract. Examination of identical specimens of serum by the methods of Wassermann, of Vernes and of Kahn discloses frequent discordant results, especially in serum from syphilitics under treatment or in a latent stage of the disease. Such observations as the few selected for the following table are not unusual.

Wassermann	*Vernes Reading*	*Kahn*	*Remarks*
—	71	4	Tertiary syphilis
—	22	—	Angina pectoris
—	0	4	Colloid goiter
—	0	4	Tertiary syphilis
4	30	—	Syphilis
3	0	4	Tertiary syphilis
4	52	—	Tertiary syphilis
2	0	4	Tertiary syphilis
4	22	—	Syphilis

Furthermore, in testing the blood of the same patient at intervals it is found that the reading by one test may ascend while that of another may be lowered.

In all three methods the reading is an attempt to express the quantity of lipoidophilic reagin present in the patient's blood, which will enter into reaction with alcoholic extract of heart muscle. The discordant results here recorded indicate that the syphilitic reagin is not a single substance but probably a mixture of various substances, whose quantitative relations may determine a high reading when tested by one method and a low reading by another.

It is suggested that variations in composition of the reagin mixture may depend not only upon the stage of the disease but also upon the character of the tissues in which lesions are situated.

Discussion

(Dr. A. B. Wadsworth, Albany, N. Y.) I was very much interested in Dr. MacNeal's report because it confirms in many respects the experience we have had in comparing the work done in different laboratories. Although we have not worked with the Vernes or Kolmer tests, we have recently had some experience with the Kahn precipitation reaction, which indicates that the test is not quite so reliable as the complement-fixation test with the cholesterinized antigen, unless the latter is too delicately adjusted. If one compares the reactions obtained with the Wassermann tests and Kahn tests made in different laboratories, it will often be found that quite discrepant results have been secured due to technical errors and variations in the adjustment or control of the tests. We have found it particularly essential in our comparisons to be sure that the test with the cholesterinized antigen is not too sensitive and yet is sufficiently delicate. The only way I could rely upon the test being properly adjusted was

by studying the results of comparative tests made in a number of laboratories, which, from time to time, have been asked to coöperate. Such a comparison gives one a valuable basis for the evaluation of the accuracy of serological examinations. If the results are plotted, here and there one laboratory will miss a reaction, but those obtained by the majority furnish a very fair basis for the control of the accuracy of your own test.

(Dr. A. S. Giordano, South Bend, Ind.) I have tried the Kahn, Kline, and Kolmer tests and even though this is rather preliminary, comparing it with the Kolmer, the Kline is far superior to the Kahn and runs more parallel to the Kolmer than the Kahn. There is 10 or 15 per cent difference in the two.

(Dr. MacNeal, closing.) We have taken special precautions to avoid confusion of specimens and technical errors, which are obviously suggested by such discordant results. I am myself convinced that there is something else besides technical error involved here. I would like to decry the too great faith in any one serological test or any group of serological tests. On the whole, it seems to me the syphilologists are putting too much confidence in serological examinations. Some men will not use them at all but they are unwise in this. Serological tests are a great help when used in the proper way. The positive Kahn, positive Wassermann or positive Vernes reading may be regarded as absolute proof of syphilis by enthusiasts so that they tend to neglect other evidence. One should not be so slavishly enthusiastic about any one method. A proper clinical record including the results of serological tests from time to time is still the best guide.

THE DIFFERENTIATION BETWEEN INFECTION AND IMMUNITY BY SERUM AGGLUTININ ANALYSIS. I. Forest Huddleson (by invitation), East Lansing, Mich.

Abstract. A method is described for differentiating serum agglutinins occurring in the host during the presence of a pathogen, from those which may be demonstrated in the serum after the passing of the inciting agent, or arising as a result of preventive inoculation.

The differentiation of serum agglutinins by the described method depends upon the following factors: the employment of a specific antigen suspended in 12 per cent sodium chloride solution and the addition of the antigen to 0.1 cc. of serum on a glass plate without mixing. The differentiation is determined after a lapse of one minute by the type of flocculation.

The method has been applied to the study of Bang's abortion disease in the cow and undulant fever in man.

Discussion

(Dr. A. S. Giordano, South Bend, Ind.) I would like to ask Dr. Huddleson if he would make some statement about the preparation of antigen. This is a very important question when applied to humans, particularly when doing routine agglutination tests on blood. We are often confronted with the question as to the determination of agglutination, whether or not the patient has had the disease, or is at present affected by the disease. We would like to ask him whether or not the antigen is the same as he uses in making agglutination tests as described in his previous article.

(Dr. Huddleson, closing.) The antigen for the differential test is prepared very much like the antigen for the rapid macroscopic agglutination test described in a recent number of the *Journal of Infectious Diseases*, the chief difference being the elimination of the boiling procedure.

STUDIES ON THE ANTIGENIC PROPERTIES OF ULTRAMICROSCOPIC VIRUSES. E. W. Schultz, Stanford University, California.

Abstract. Though specific neutralizing antibodies were easily demonstrable in antisera experimentally produced in rabbits against the viruses of vaccinia, rabies and herpes, no evidence whatever of specific complement fixing or precipitating antibodies could be elicited in these sera. These facts together with the general trend of the results already reported in the literature on these and other ultraviruses, raise the question of whether perchance the ultraviruses, because of their relatively low physical and chemical organization, as suggested by recent ultrafiltration studies, may not, like the true toxins, exhibit antigenic properties of a more restricted and specialized character than those which we know to hold, for example, for bacteria and other complex proteinaceous bodies.

Discussion

(Dr. John Reichel, Philadelphia.) I would like to ask Dr. Schultz how he determined the rabicidal properties of the immune serum.

(Dr. Schultz.) A suitable suspension of rabies virus was brought together with the inactivated immune serum in a test tube and incubated at 37.5° C for four hours. This mixture was then injected intracerebrally into a rabbit. At the same time a control rabbit received a similar injection of the virus treated in an identical manner with inactivated normal rabbit serum.

(Dr. Reichel.) I am interested in the fact that neutralization of the virus was obtained with inactivated immune serum. In some studies which I have made I have been able to demonstrate rabicidal action only when I used fresh serum. Inactivated serum had no effect on the virus.

(Dr. Schultz, closing.) We obtained definite rabicidal action with *inactivated* immune sera. That rabicidal action may be obtained with *inactivated* immune sera, is, I believe, also quite well supported by the literature.

ON THE MECHANISM OF OPSONIN AND BACTERIOTROPIN ACTION. I. EXPERIMENTS WITH ACID-FAST BACTERIA. Stuart Mudd, Baldwin H. Lucké, and (by invitation) Morton McCutcheon and Max Strumia, Philadelphia, Pa.

Abstract. These experiments represent the first stage of a study directed toward analysis of the mechanism of opsonin and bacteriotropin action in physical-chemical terms. They are intended to answer the question: "What changes do sera effect in acid-fast bacteria in preparing them for phagocytosis?"

Rabbit-immune sera have been prepared against a variety of acid-fast bacteria. The bacteria, sensitized by serial dilutions of homologous and heterologous antisera, or by normal sera, have been studied in the following reactions. (1) The bacteria remain in serum dilutions over night and the *agglutination* is then read. (2) The serum-bacterial mixtures are strongly centrifugated and the sediment resuspended by shaking; this *resuspension* reaction gives an estimate of the cohesiveness of the bacteria. (3) The sensitized bacteria are washed and their wetting or interfacial tension properties are then estimated in the *interface* reaction. (4) The electrical charge of the washed, sensitized bacteria is determined by *cataphoresis*. These four reactions together give a picture of the surface properties of the bacteria. (5) Meanwhile mixtures of rabbit leucocytes with (*a*) washed, sensitized bacteria, or with (*b*) bacteria and serum dilutions, are rotated in stoppered vials on a Robertson agitator. Smears are made from each mixture, stained, and a hundred leucocytes in each smear are observed

microscopically. The percentage of leucocytes which have taken up bacteria is recorded.

Immune sera reacting with acid-fast bacteria cause agglutination, phagocytosis, increased cohesion of the bacteria, reduced bacterial surface charge, and change from a bacterial surface readily wet by oil to a surface readily wet by water. The most important relationship thus far brought out is that in practically every instance in which an immune or normal serum has caused increased phagocytosis it has caused in approximately corresponding degree the above-indicated changes in surface properties. Similarly when immune sera have reacted with the bacteria so as to produce surface changes, corresponding increase in phagocytosis has usually resulted. Thus, of twenty-one series in which bacteria were treated with homologous sera, nineteen showed surface changes and bacteriotropin action in roughly corresponding degree; of sixteen heterologous series there was satisfactory correspondence between surface changes and bacteriotropin action in twelve.

Failure of correspondence was due to failure of bacteriotropic action with old sera (approximately 18 months ageing). Another anomaly was observed with these aged sera. Striking phagocytic prezones were regularly obtained when bacteria were treated with aged sera homologous with them; phagocytosis reached a maximum in some instances, not as with fresh sera in the highest serum concentrations, but in serum concentrations as low as one tenth of one per cent.

The serum of four rabbits has been followed by similar tests at weekly intervals during the course of active immunization. Striking correlation was found between the bacterial surface changes and the bacteriotropin effects produced by the sera of these animals.

The correlation between bacterial surface changes and opsonin effects produced by fresh normal rabbit sera was satisfactory with eleven out of twelve sera. When the sera were heated for 30 minutes at 56° C their effects on the bacterial surfaces were reduced but by no means abolished; on the other hand their opsonic effects were lost in nine out of sixteen cases. Normal human sera altered the bacterial surface properties but did not induce phagocytosis by rabbit leucocytes.

In summary, then, sera which in these experiments have increased the phagocytosis of bacteria have concomitantly increased the cohesion, decreased the surface charge and altered the wetting properties of the bacteria. Similarly, but with certain exceptions, when sera have altered the surface properties of bacteria as indicated they have increased phagocytosis. The exceptions have practically all been with aged or heated sera.

Discussion

(Dr. R. R. Mellon, Pittsburgh.) I would like to ask Dr. Mudd whether these organisms are sensitized and consequently reduced in charge or whether the flocculation found occurred spontaneously.

(Dr. Mudd.) The cohesive factor seems to be more important in affecting agglutination and seems to be more variable with acid-fast bacteria than with other species. Flocculation in our experiments has not been spontaneous but has been with sensitized bacteria whose surface potential difference has been reduced and whose cohesiveness has been increased by sensitization. Because of the difficult and irregular agglutinability of these strains we do not regard the ordinary agglutination reaction as of much importance with them. By centrifu-

gating the sensitized bacteria and then resuspending them, however, a fair estimate of their relative cohesiveness in the various serum dilutions can be obtained.

(Dr. Mellon.) Would you say, then, that they are perfectly sensitized?

(Dr. Mudd, closing.) I think that probably the data indicate that the film of antibody proteins is possibly incomplete, that it is not a complete film.

AQUEOUS–LIPOIDAL PHASE REVERSAL IN THE CELL WALL OF 'S' AND 'R' Bacterial FORMS: ITS BEARING ON THEORIES OF ELECTRICAL P. D. IN SUSPENSION STABILITY. Ralph R. Mellon, Pittsburgh, Pa.

Abstract. The large number of studies on the electric charge of bacteria especially in its relation to spontaneous agglutination have never been able to evaluate the rôle of the surface tension factor. This, because of no satisfactory method for measuring bacterial interfacial tension, the method advocated by Northrup and DeKruif being quite inaccurate. The result has been an undue appreciation of the charge factor at the expense of the surface tension factor.

Several years ago one of our papers called attention to this aspect of the situation. The ground for our position rested on experiments with sodium oleate whereby certain strains of high interfacial tension could be readily emulsified with this reagent. In this connection we also showed that the principle of ion antagonism was applicable, which indicated the presence of an aqueous-lipoid system in the cell wall that was reversible by the cations Na and Ca where the anion Cl remained the same.

This contention is now susceptible of experimental demonstration with certain strains, *in which instances* the electric charge appears to play a minor or even an insignificant rôle in suspension stability. In a word, the experiments are as follows: The Ca ion brings the lipoid phase to the external surface of the bacterial cell wall, as can be shown by the fact that the organism will pass from an aqueous solvent to lipoidal solvents. The effect of the Ca appears to be a maximal one, and the effect of the solvent a quantitative one, depending on the relative solubility of the lipoid in the aqueous solvent. On the other hand, in the presence of the Na ion alone, *under no known circumstances* will the organism leave an aqueous menstruum for a lipoidal one.

These results assume a heightened interest in the light of the recent work of March, who concludes essentially that the greatest electrical P. D. ever recorded with colloidal particles is less than one-twentieth of that necessary to bring about significant mutual repulsion of particles similarly charged.

Discussion

(Dr. S. Mudd, Philadelphia.) Northrup and DeKruif made an important advance in analysis of the mechanism of agglutination when they showed that it depended upon two factors: a surface electrical potential difference tending to prevent collisions between the bacteria and a cohesive force tending to hold them together once they have collided. It would seem a step backward to try to eliminate consideration of the surface potential difference and explain the flocculation only in terms of cohesion as surface tension. Moreover, a surface largely lipoidal does not necessitate spontaneous agglutinability. Many of the acid-fast strains have a surface rich in lipoid and yet form stable suspensions in 0.85 per cent NaCl solution.

(Dr. Mellon, closing.) I am sorry that I stressed this thing enough to give Dr. Mudd or anyone else the impression that I was holding any special brief

for surface tension effects to the entire exclusion of other surface factors, such as potential differences. The experiments here presented are intended first to emphasize a fact apt to be forgotten, *viz.*, that there is no method for measuring the interfacial surface tension of a bacterium — therefore when we draw conclusions from potential difference observations we must always assume that the surface tension factor is behaving in accord with the stipulations of Northrup and DeKruif. But it so happens that in this strain we have an organism where the interfacial tension is so high as to be revealed by the solubility methods employed. If we were not aware of this fact, we should conclude that the drop in P. D. with $CaCl_2$ below the critical level explained the agglutination. Now since the action of the $CaCl_2$ was essentially to bring the lipoid to the surface of the organisms, thus virtually converting them into oil droplets, why should we invoke potential difference as an explanation, at least any more than we should invoke it to explain why oil and water do not mix? This is not inconsistent with the view that the P. D. action is a dispersive one, but under conditions such as the above, it would needs be many times greater than the measurements to be taken seriously.

OBSERVATIONS ON THE INTESTINAL BACTERIOPHAGE IN THE SPECIFIC INFECTIOUS DISEASES. D. M. Cowie and (by invitation) Henry G. Poncher, Ann Arbor, Mich.

(*Abstract not received.*)

IMMUNOLOGICAL STUDIES IN RELATION TO THE SUPRARENAL GLAND. David Perla and (by invitation) J. Marmorston-Gottesman, New York City.

Abstract. Hemolysin formation in normal and suprarenalectomized rats was studied. A single intraperitoneal injection of 1 cc. of a 10 per cent suspension of sheep cells in normal adult albino rats results in an optimum high hemolysin titer five days after injection, which gradually falls, disappearing at about the third week. Ten times this amount produces lower hemolysin titers.

Bilateral suprarenalectomy in rats subsequently injected intraperitoneally with 1 cc. of a 10 per cent suspension of sheep cells at various intervals after operation (48 hours, 7, 14 and 28 days) resulted in a depression of hemolysin titer during five weeks following the operation, the depression being most marked during the first week. Bilaterally suprarenalectomized rats injected intraperitoneally two weeks after operation with 1 cc. of undiluted sheep cells have hemolysin titers higher than do normal rats. The quantity of antigen necessary to yield the maximum titer in suprarenalectomized rats two weeks after operation is ten times the quantity necessary to yield the same titer in normal rats. Traumatization of the perisuprarenal tissue in rats produced the same effect on the antibody-forming capacity as suprarenalectomy.

Further, the effect of adrenalin on hemolysin formation in normal rats was studied. Injections of adrenalin in amounts of 0.4 mg. per kilo rat per day in divided doses in normal rats for three days prior and four days subsequent to injection of 1 cc. of a 10 per cent suspension of sheep cells, produced a marked depression in hemolysin formation. The same daily amounts of adrenalin injected during four days subsequent to the sheep cell injection produced a depression to a lesser degree. Adrenalin injected in the same daily amounts but only during one day prior and two days subsequent to the injection of red blood cells had no depressing effects.

AN EXPERIMENTAL STUDY OF THE ACTION OF *B. Welchii* TOXIN ON BONE MARROW. John C. Torrey and (by invitation) Morton C. Kahn, New York City.

Abstract. This study is in continuation of our investigation of the anemia produced in laboratory animals by a potent *B. welchii* toxin. In previous communications attention has been directed to the abnormally high *B. welchii* counts in fecal specimens from pernicious anemia cases. It has also been shown that a transitory anemia with a blood picture strongly suggestive in a number of features of the pernicious type may be produced in monkeys by intravenous inocculations of such a toxin, and further there was reported evidence of the absorption of this toxin from the gastro-intestinal tract of monkeys which had been treated with a weak solution of sodium fluoride to induce a catarrhal inflammation. The present study is concerned with the action of the toxin on the bone marrow following direct inoculation.

The *B. welchii* toxin was prepared by growing the organism in casein-digest broth in sealed tubes as described heretofore. We have found that 2 cc. of fresh rabbit blood per 30 cc. of medium may be substituted for the pigeon muscle. The twenty-four-hour culture was rendered sterile by passage through a Berkefeld filter. Under ether anesthesia 0.5 cc. of the filtrate (1 M.L.D. for a pigeon) was inoculated into the tibial marrow of each of a series of rabbits and of a monkey and the blood and marrow changes were followed for varying lengths of time. In contrast to the immunity sooner or later following one or more intravenous inoculations of the toxin, a single intramarrow injection has a progressive injurious effect causing a severe chronic anemia with very marked loss in weight and frequently a fatal issue. Also the inoculation into the marrow of one bone caused the same type of degenerative changes in almost or quite equal degree in marrows of bones far removed therefrom. In view of the fact that the intravenous inoculation of the same dosage or a series of increasing amounts of toxin would not cause marrow changes of this character or degree, it is difficult to explain the apparent whole involvement of the marrow system following toxin injury to a single bone marrow.

No animal inoculated in this way has made a recovery. Two rabbits died after 74 and 75 days respectively. Others were observed for from 100 to 112 days and were very weak and anemic after that period. Within 24 hours following the inoculation the hemoglobin and the erythrocyte count were reduced about 50 per cent. From this time on the erythrocyte count ranged from 1.5 to about 2.5 million and the Hb from 50 to 60 per cent, except at one or more periods of marked remission. About 10 days following the inoculation the color index rose above 1.0 and stayed there until about two weeks before the death of the animal. Morphological and tinctorial changes in the blood were, on the whole, not quite so pronounced as those following intravenous toxin inoculations. The attempt at blood regeneration was very strikingly shown in the case of three rabbits inoculated into the tibial marrow with 0.5 cc. of the toxin at the same time. The remission occurred in all three simultaneously and seven weeks following the inoculation. This lasted not over a few days during which the erythrocyte counts rose to nearly four million and the color indices dropped below 1.0.

As stated above, inoculation of the *B. welchii* toxin into one bone marrow causes a progressive deterioration of apparently the whole marrow system. This process starts within 18 hours with an atrophy of the marrow cells, ingress of many polymorphonuclear leucocytes and degeneration of the giant cells. After 12 days there was an extremely marked atrophy of the marrow cells, a marked increase and subsequent degeneration of the fat cells, but the most striking

feature was the great abundance of polymorphonuclear leucocytes. At the terminal stage the marrows were a much darker red color than in the normal rabbit. Microscopic examination showed there had occurred in the marrow of the long bones on both sides of the body a replacement of fat and lymphoid cells by a peculiar homogeneous granular material. The few remaining cells were mostly rather large, hyperchromatic granular mononuclears of the myelocyte type. No definite traces of forming red blood cells could be found.

Two control rabbits inoculated with 0.5 cc. of the sterile medium into a tibial marrow were almost unaffected by the treatment. After two months they had gained in weight. The blood examinations showed little or no deviation from the normal. The marrows at necropsy had the same color and appearance as in normal rabbits and microscopically exhibited no change. The medium itself was thus eliminated as a causative factor in the marrow and blood deterioration.

Discussion

(Dr. W. M. Simpson, Dayton, O.) In view of the fact that the reaction of the bone marrow to *B. welchii* toxin is not a local one, it would seem that the entire reticulo-endothelial apparatus might be similarly involved. I would like to know what changes occurred in the spleen and liver; whether there was reticulo-endothelial hyperplasia and hemosiderin deposition?

(Dr. Kahn.) I have nothing further to add to Dr. Simpson's remark. I made no further observation on the spleen or other organs and I cannot give any further definite information.

(Dr. Ottenberg, New York City.) If it is injury to bone marrow, how can one explain the very sudden drop in 24–48 hours of the hemoglobin of the red cells.

(Dr. E. E. Ecker, Cleveland.) I would like to ask whether or not these reactions are specific or non-specific?

(Dr. Kahn, closing.) There is undoubtedly some hemolytic activity in the blood system after intravenous injection. You will get a marked drop in erythrocyte count after injection into the blood stream or the marrow. As far as the production of macrocytes is concerned, marked variations in the size of the erythrocytes were induced by injecting *B. welchii* toxin into the marrow and round to oval macrocytes were frequently observed. As far as other anaerobes are concerned, changes induced by injection of *B. tetanus* toxin were not as marked as those by the *B. welchii* according to the reports of other observers. Also results with staphylococcus were not as apparent as those with *B. welchii*. No observations on reticulated erythrocytes were made.

EXPERIMENTAL INOCULATION OF CHICKENS WITH LYMPH NODES OF HODGKIN'S DISEASE. Elise S. L'Esperance, New York City.

(*Abstract not received.*)

STUDY ON LONGEVITY OF DIPHTHERIA CULTURES. Henrietta Calhoun, Rockford, Ill., and Henry Albert, Des Moines, Iowa.

(*Abstract not received.*)

IS LYSIS OF BACTERIA AN ESSENTIAL PART OF THE PHENOMENON OF BACTERIOPHAGY? J. Bronfenbrenner and (by invitation) D. Hetler, New York City.

Abstract. At the meeting of this Society last April, we presented experimental evidence suggesting that lysis of bacteria in the presence of phage may be the direct result of the rupture of bacterial cells, due to an increased uptake of

water from the medium. If this conception is correct, one should expect that in all cases where the swelling of bacteria is prevented, there should be no lysis, and conversely, whenever, under the conditions of the experiment, lysis in the presence of phage is prevented, there should be no swelling of bacteria. These predictions were tested experimentally, taking advantage of the fact that under certain conditions lysis in the presence of phage can be prevented at will.

Susceptible bacteria were seeded on the surface of solid media of different composition, and subsequently droplets of active phage were deposited at various places on the seeded surface. At suitable intervals, contact impressions were taken from the spots on which bacteriophage was deposited. It was found that whenever lysis took place, bacteria invariably underwent swelling. Conversely, all contact impressions taken from spots where phage was deposited, but where lysis did not take place, showed that the bacteria failed to swell.

Thus far, we have attempted to prevent lysis by means of (1) addition to the medium of antibacterial sera; (2) subjecting the cultures to the effect of high temperatures (42–45° C), and (3) increasing the concentration of agar or gelatin in the medium.

The mechanism responsible for inhibition of swelling and visible lysis in the first two instances remains for the present unknown. From the fact, however, that bacterial growth under the droplets of phage failed to show any evidence of being affected by its presence, we are inclined to consider that bacteria themselves have undergone some change under the conditions of the experiment, whereby all action of phage on them was excluded. The failure of bacteria to swell and undergo visible lysis on media containing high concentrations of agar or gelatin, we think is directly due to the competition for water between the medium and the bacteria. In this experiment phage undoubtedly exerted its effect on bacteria (even in the absence of lysis) as evidenced by the fact that bacterial growth over the spots where phage was placed greatly exceeded that present on the surrounding medium. Furthermore, when the portions of medium on which phage was deposited were cut out and the amount of phage present in them was determined, it was found that it had undergone some increase in concentration, as compared with the control. (Phage deposited on sterile medium.) It appears that in addition to showing the intimate relation existing between the swelling and the subsequent lysis of bacteria, these experiments confirm earlier observations concerning the regeneration of phage in the absence of lysis. However, the regeneration of phage in these experiments was only partial, and in no instance approached the rate of regeneration observed by us in similar experiments on the media containing lower concentrations of phage or gelatin, and therefore the possibility that some individual bacteria may have undergone lysis cannot be excluded.

The conclusions we draw from these experiments are: that lysis is not an essential part of the phenomenon of bacteriophagy; that under certain experimental conditions the presence of phage is plainly demonstrable in the absence of lysis; that lysis proper is the direct result of the uptake of water by the bacteria, and that whenever this uptake of water is interfered with, either through the changes induced in the bacteria themselves, or in the medium, the swelling and lysis of bacteria are prevented.

Discussion

(Dr. J. W. Jobling, New York City.) I should like to ask Dr. Bronfenbrenner if he varied the salt content of his bacteriophage; if that in itself would have the

same effect as growing the organisms on the media, in other words, change the osmotic effect in that manner?

(Dr. Bronfenbrenner, closing.) It has been observed that when bacteria are grown on media containing high concentration of salts, they contain correspondingly high concentration of salts in their protoplasm. Such bacteria undergo rapid swelling and lysis when transferred to highly dilute media. Inversely, it may be expected that if swollen bacteria, as those in our experiments, are transferred to media containing high concentration of salts they will lose water and will not burst. We tried to carry out such experiments using various salt and sugar solutions, but did not obtain conclusive results. Such experiments are very difficult to perform due to the fact that swelling of bacteria and particularly the bursting of swollen bacteria occurs very suddenly and rapidly.

THE RESORCIN FLOCCULATION TEST FOR ACTIVITY OF TUBERCULOSIS. Adelaide B. Baylis (by invitation) and Ward J. MacNeal, New York City.

Abstract. The blood serum during active tuberculosis is altered in such a way that various reagents will produce a cloud in it. Daranyi employed ethyl alcohol for this purpose. More recently various phenols have been shown to react with tuberculous human serum to produce a flocculation. Vernes employs a 1.25 per cent solution of chemically pure resorcin in doubly distilled water, mixing this with an equal volume of the patient's serum and reading the optical opacity in his photometer at once and again after four hours at 25° C. The difference between the two readings is less than 15 in normal persons, from 15 to 30 in relatively quiescent tuberculosis and above 30 in actively progressive tuberculosis. The test is not specific in a bacteriological sense. In fact, high readings are found for a short time in the primary stage of syphilis and frequently in advanced neoplastic disease. We have also observed high readings in active pneumonia and in pulmonary abscess.

More recently, Baylis has devised a simple technic which does not require the photometer. The resorcin solution and the serum are mixed by pouring from one test tube to another and the mixture is left in the room four hours and then overnight in the refrigerator. The visible precipitate is read roughly and recorded as minus, plus minus, plus, two plus, three plus, or four plus. It is also possible to distinguish an atypical flocculation in which the flakes are coarse and voluminous. This atypical reaction appears to be associated with other conditions than tuberculosis, especially with pulmonary abscess and advanced malignant neoplasm.

These tests promise to be of considerable value in measuring the activity of a known tuberculous process and, when due regard is given to their limitations, they are of some value in differential diagnosis.

THE FATE OF TUBERCLE BACILLI IN VARIOUS ORGANS OF THE RABBIT. Max B. Lurie (by invitation), Philadelphia, Pa.

Abstract. In this study an attempt was made to follow the fate of tubercle bacilli in the lung, liver, spleen, kidney and bone marrow of rabbits infected with large and small doses of human and bovine tubercle bacilli intravenously by determining the number of colonies of tubercle bacilli that can be recovered from comparable quantities of tissue on egg media at varying intervals during the course of infection.

It was found that the original distribution of the bacilli to the several organs

follows the distribution of particulate matter, namely, in the following order: spleen, liver, lung, bone marrow and kidney per gram of tissue.

At first the tubercle bacilli, both of the human and bovine types, grow in all the organs without any effective opposition. The rate of growth of both types of bacilli differs greatly in the various organs. The human type of tubercle bacillus grows faster in the several organs than does the bovine type. This initial growth is followed by a more or less complete destruction of the bacilli in all the organs of the rabbits infected with the human bacillus, while in the bovine infected animals the destruction is more or less complete in the liver, spleen and bone marrow and multiplication continues without effective opposition in the lung and kidney until the death of the animal. The time at which destruction appears in the several organs depends on the organ in question, the type of bacillus used, and the infecting dose. The liver, spleen and bone marrow show the earliest destruction. The lung and kidney destroy the bacilli later if at all. Destruction is apparent earlier in the human infection and later in the bovine infection. In rabbits infected with the human type of bacillus the destruction in the liver, spleen and bone marrow is brought about earlier with a large dose and later with a small dose. The degree of destruction in a given time is more complete in the several organs in rabbits infected with the human type than with the bovine type in the same dose. With the same type of bacillus the destruction is more complete in a given organ with a large dose than with a small dose. This is especially true of the spleen.

It is suggested that the virulence of a certain type of mammalian bacillus for the rabbit is to some extent inversely related to the rate of its initial growth. The human type grows faster in the body of the rabbit and brings about an earlier altered body reaction and destruction of the organism than does the more slowly growing bovine bacillus.

The destruction in the various organs, after the altered body reaction has been brought about, is rapid at first and progresses more slowly as time proceeds. So that even six months after the intravenous infection of rabbits with small doses of human tubercle bacilli they have not yet completely disappeared from the lung and spleen, while they can no longer be isolated from the liver, bone marrow and kidney.

Some evidence is presented that this acquired resistance to tuberculosis in the various organs is an increment of the natural resistance of the same organs to the tubercle bacillus.

Discussion

(Dr. Paul Lewis, Princeton, N. J.) I am very much interested in the presentation of this subject, which is really extraordinarily difficult to approach. Dr. Lurie has done a great deal of work for which he deserves a great deal of credit, and has approached the matter as one who has more data. I wish to ask a question which he has not touched upon. I inoculated a series of rabbits with virulent bovine and human bacilli, and we examined the lungs particularly with great care microscopically, and the results seemed in a sense to be conclusive in that at each period up to the seventh day the number of bacilli found in the bovine and human were very difficult to discriminate, as Dr. Lurie has shown, but where Dr. Lurie's results seem to show that there is a rapid and progressive multiplication microscopically. On direct examination nothing such appears with the human strain. This bacillus disappears after 7, 8 or 10 days except possibly in foci which are not related to any general condition in the lung and

these also in some rabbits and not in others, whereas the bovine seems to develop as if it were on culture media. The human bacillus is ordinarily regarded as much easier to cultivate. It is possible that these observations are not so unharmonious because of the culture adaptability of one strain more than the other. Ability to grow is certainly not easy to harmonize with our previous observations and one has to be a little slow to accept it as a general thing. The conclusions are very interesting and he is to be commended on the systematic and painstaking way he has carried on the subject.

(Dr. Lurie, closing.) In regard to the first question: I wish to state that I have not as yet completed the study by any means, and that this is not a final statement, but a suggestion of the general nature of the facts. These studies will be continued by microscopic methods. I am not sure, but it appears that the isolation of tubercle bacilli by cultural methods does not necessarily mean that they will be found by microscopic technique. We shall come back to this.

The second question is a very pertinent one. It is more difficult to isolate bovine than human tubercle bacilli from infected tissues. However, I was conscious of this difference from the very first. As a matter of fact, from two suspensions of human and bovine tubercle bacilli each containing the same amount by weight, fewer colonies of bovine tubercle bacilli will be recovered than human. If, however, tissue suspensions are added to the bacillary emulsions, it seems that you would recover similar numbers from bovine and human. Still this is only a preliminary statement. But the point I wish to stress is not the actual numbers of tubercle bacilli recovered but the relationship between them. The relative rise is much slower in the bovine than in the human.

With B. C. G. some work has been done where I found essentially comparable results, namely, a still more rapid growth than the human type followed by a still more rapid destruction.

THE BLOOD IN HOG CHOLERA. Paul A. Lewis, Princeton, N. J.

(*Abstract not received.*)

THE INFLUENCE OF VITAMINE B AND SUBSTANCES CONTAINED IN BLOOD ON THE LAG PHASE OF TISSUE CULTURES OF THE CHICK SPLEEN. F. A. Hecker (by invitation), Ottumwa, Iowa.

Abstract. The ten-day chick spleen when planted in a mixture composed of one part of chicken plasma, one part of normal salt solution or Ringer's solution, and one part of potato broth, shows the growth of an eighteen-hour culture in this mixture is markedly increased, when compared to a control culture planted in one part of chicken plasma and one part of normal salt solution or Ringer's solution. In addition to the foregoing experiments, some of the substances contained in blood, namely urea, uric acid, etc., were added in definite amounts to the normal salt solution or Ringer solution and were mixed in the same volume proportionally as just described. No inhibitory influence on the growth of the cells could be detected when some of them were employed. This observation confirms the work of Burrows, namely, that high concentrations of Vitamine B induce active cell growth.

THE EFFECTS OF AMNIOTIC FLUID ON SEROUS SURFACES. Shields Warren, Boston, Mass.

Abstract. The chemical study of amniotic fluid indicates that it is a combination of transudate from the maternal blood and secretion from the fetus. One

apparent function of the amniotic fluid is to prevent adhesions between the fetus and membranes.

When amniotic fluid is added to the peritoneal cavity of animals which have been operated on so as to produce adhesions it prevents entirely their formation in nearly one-half the cases, and materially reduces the number of adhesions in others. In only a few cases has it been a complete failure. The controls have uniformly shown dense adhesions.

This action is apparently due to two factors: (1) shortening the time of oozing from injured surfaces; (2) the lubricating effect of the presence between eroded surfaces of a fluid which is not rapidly absorbed. There appear to be no ill effects from using the amniotic fluid of one species in animals of another species. Amniotic fluid does not delay wound healing nor affect the rate of growth of fibroblasts in tissue culture.

The sterility of amniotic fluid as such is difficult to maintain. Consequently, a method of alcoholic fractional precipitation has been developed which gives a concentrated fraction of amniotic fluid which is practically as efficacious as the original fluid. If anything, those animals receiving amniotic fluid have shown less shock and smoother convalescence than the controls. At present the concentrate is prepared from the amniotic fluid of the outer sac of the cow. No ill effects have been noted from its use either in animals or in the limited number of human cases in which it has been tried.

Experiments are under way at the present time to determine the efficacy of amniotic fluid concentrate in joint cavities, the pericardial and pleural cavities, and the conjunctival sac. It may be of value in relieving the symptoms of fibrinous pleuritis or pericarditis.

The amniotic fluid concentrate as prepared at present contains a small amount of mercurial antiseptic, and it is to be hoped that in cases of peritonitis this fluid may be of advantage when combined with a larger amount of antiseptic. It has definite advantages over other substances which have been tried for the purpose of preventing formation of adhesions in that it does not induce a foreign body reaction, it does not act through digestion or destruction of tissue, and it does not interfere in any way, so far as can be determined, with normal function.

INTRA- AND SUPRAVITAL STUDIES OF FIXED AND CIRCULATING CELLS IN PERITONITIS. Bernhard Steinberg, Toledo, Ohio.

Abstract. Peritonitis was produced by intraperitoneal injection of colon bacilli suspended in gum tragacanth and by intraperitoneal injection of fecal material. Leucocyte counts were taken prior to the production of peritonitis. Following the production of the infection, counts were made at intervals of one-half and one hour. It was found that within two to three hours after the onset of infection, the leucocyte count in the peripheral blood dropped to one-half or one-third of the pre-infection number. Within six to seven hours, the number of leucocytes returned to the level prior to the production of the peritonitis. Following this period, the number of leucocytes rapidly increased and a marked leucocytosis occurred. This part of the experimental work showed that following the onset of the peritoneal infection, there was a marked drop in the leucocytes in the peripheral blood. The leucocytosis observed in infections apparently does not ensue for nine to ten hours after the onset.

Studies of the peritoneal smears during the course of acute peritonitis showed that the exudation of free cells occurs in non-immunized animals approximately three hours following the onset of the infection. The appearance of the free

cells was shortly after the drop of the leucocytes in the peripheral blood. The wandering cells (those found in the general circulation) were in predominance in the peritoneal exudate during the first five to six hours in the course of the peritonitis. Coincident with the rise of leucocytes in the peripheral blood, monocytes predominated in the peritoneal fluid. The monocytes were differentiated by the supravital staining with neutral red. Following the appearance of monocytes in the peritoneal exudate a small number were found in the peripheral blood. In immunized animals, the peritoneal exudate showed appearance of cells within one hour of onset of infection. Many clasmatocytes were found in the exudate. Bacteria in immune animals were rapidly clumped and phagocytized by monocytes and polymorphonuclears. Non-immune animals did not show clumping of bacteria.

Study of tissue at approximately two-hour intervals during the course of peritonitis showed in non-immune animals a slow cellular response. In those animals, the mononuclears predominated in the first several hours. The first reaction of the peritoneal tissue was distension and congestion of capillaries and smaller blood vessels. Following this was the appearance of polymorphonuclears within blood vessels. Within seven hours after the onset of peritonitis a number of polymorphonuclears and approximately an equal number of mononuclears were seen in the tissue. In the immune animals, the reaction was predominately polymorphonuclears.

Immune animals showed clasmatocytes in the peritoneal exudate. Apparently, the immunity in the animal was coincident with the appearance of those cells. Clasmatocytes were not found in non-immune animals. They were found, however, in small numbers in animals which were recovering from the infection. Immune animals show a more rapid and a greater cellular reaction both in the tissue and in the exudate.

Discussion

(Dr. H. L. Jaffe, New York City.) There are, as we know, difficulties in the process of staining cells. If you permit them to stand under the microscope for a period of time, because of the changes that take place in these cells or as the result of injury the type of granule varies considerably. So I feel that Dr. Steinberg should take a little caution in interpreting the character of cell he finds in the peritoneal exudate, because certainly a great number of these cells are injured at the time he stains them supravitally.

(Dr. Steinberg.) I did not have time to present many of the methods employed in the experiments. The cells were examined immediately after removal from the peritoneal cavity, as a rule within one minute.

(Dr. Jaffe.) I meant injured at the time of removal.

(Dr. Steinberg, closing) If Dr. Jaffe means that the cells were already injured in the peritoneal cavity prior to removal, that is very probable, but it was possible to differentiate the types of cells, even though injury is to be admitted. I appreciate that injured cells may show altered capacity to dye intake but again we are dealing with constant factors; it is difficult to assume that in repeated experiments, properly controlled, only certain types of cells were injured and others exposed to the same conditions remained uninjured.

CELLULAR RESPONSE IN RABBITS TO REPEATED INJECTIONS OF NON-HEMOLYTIC STREPTOCOCCI. Robert N. Nye, Frederic Parker Jr., and (by invitation) David Seegal, Boston, Mass.

(*Abstract not received.*)

THE RHEUMATIC NODULE. B. J. Clawson, Minneapolis, Minn.
(Abstract not received.)

THE CERVIX UTERI AS A FOCUS OF INFECTION IN CHRONIC ARTHRITIS. L. W. Famulener and (by invitation) Frederick J. Matthews, New York City.
(Abstract not received.)

EARLY VISCERAL LESIONS OF TULAREMIA. Walter M. Simpson, Dayton, Ohio.

Abstract. A complete autopsy on the most rapidly fatal case of tularemia of which there is any record (five days) afforded an opportunity to study the histopathological characteristics of the early lesions of this disease. The increasing importance of this disease, both clinically and pathologically, is manifested by the fact that the writer unearthed forty-nine cases of this disease in Dayton, Ohio, during a brief period of four months.

The axillary and epitrochlear lymph nodes showed multiple recent areas of focal necrosis, miliary and submiliary in size. Surrounding the focal necroses there was early fibroblastic and epithelioid proliferation with a remarkable hyperplasia of the reticulo-endotheliocytes. The granulation tissue was entirely devoid of new-formed capillaries. Giant cells of the Langhans type, which have been described in practically every case previously reported, were absent, although some of the histiocytes had fused, forming a kind of symplasm, probably representing early giant cell formation. The normal architecture of the lymph nodes was destroyed.

The spleen showed similar focal necroses involving by far the greater part of the spleen. The diffuse reticulo-endothelial hyperplasia was more marked in the spleen.

The hepatic lesions varied markedly from the lymph node and splenic lesions. There was practically no caseous necrosis, the lesions being entirely made up of foci of reticulo-endothelial hyperplasia. In the right lung were two cherry-sized areas of caseous necrosis with epithelioid and fibroblastic proliferation and polymorphonuclear infiltration at the periphery.

The lesions described herewith should be classed with the infective granulomata, and are obviously earlier lesions of the disease than have been described heretofore.

Discussion

(Dr. H. H. Permar, Pittsburgh.) The lesions shown here by Dr. Simpson strike me as the most acute possible type of granuloma. I remember some subcutaneous lesions in a case of a much more chronic type in which we had marked hyperplasia of the vascular endothelium, giant cells, and all the features of a granulomatous reaction. The necrotic areas, it seems to me, looking at the lantern slides, show a certain number of polymorphonuclears among the necrotic tissue. I would like to know if that is correct. We found numbers of them in our sections in the same locations.

(Dr. E. R. LeCount, Chicago.) I should simply like to place on record that Dr. S. John House of Nashville, Tenn., showed me during the past winter microscopic preparations of human tissues with this disease and that we discussed the similarity the lesions possess to bubonic plague and others of the so-called hemorrhagic septicemia group. We also discussed the hazards of postmortem examinations of persons who died from these diseases. I believe they are very considerable, probably more than the average streptococcus infection. I have

no doubt the necroses shown us here in the lantern pictures contain a large amount of fibrin.

(Dr. G. R. Callender, Washington.) I would like to congratulate Dr. Simpson on having the earliest case of tularemia reported. Lesions of the type he has shown cannot be differentiated from the focal necroses of other septicemias. Those he has shown today approach the characteristic pathology seen in the guinea pig, and in some instances the pathology seen in the mouse. Even in this early lesion there is hyperplasia of the reticular cells. I believe those lesions are all too early to give rise to the response of giant cell formation. One thing about those focal necroses: a section through the outside of an area will show almost a pure endothelial proliferation, but if you make serial sections you will usually find that there is a beginning necrosis even in relatively small lesions.

(Dr. Francis, Washington.) Dr. Simpson has done something that is as fine as could be done. He has studied a number of cases in Dayton, Ohio, for the last two years. He diagnosed his cases by examining the blood and found the organism in the blood.

(Dr. Simpson, closing.) In many of the foci of the liver, we did make serial sections of these lesions and there was no extensive caseation present. As regards the danger of acquiring the infection from autopsies, it is, of course, very great. Twenty investigators have been victims of laboratory infections. We autopsied more than 100 animals during the course of this study, but we employed extraordinary precautions throughout. Nobody in our laboratory acquired the disease.

STUDIES IN THE PATHOLOGY OF SCHISTOSOMIASIS IN PORTO RICO (*S. Mansoni*). Robert A. Lambert, San Juan, Porto Rico.

(*Abstract not received*)

UNUSUAL TUMOR OF THE KIDNEY. Alfred Plaut, New York City.

Abstract. White female, 59 years old. Removal of kidney stone, formation of a sinus; swelling in scar three months after operation. Persistence of sinus, increase of swelling; pyelogram indicates pyonephrosis. Nephrectomy one year after removal of stone. Gross specimen from outside looks like pyonephrosis. The calices are much dilated, no kidney tissue found, the outline of kidney is fairly normal. The calices are filled with thin brown fluid; much gelatinous material covers the inner surfaces, partly contained in small cysts. Innumerable small papillary protrusions cover the greater part of the calices. Similar papillae are seen in the ureter. The ureter is in open communication with the calices. The microscopic picture is mostly that of mucosa of large intestine. There are typical mucus producing glands, a loose propria, some lymph follicles and smooth muscle. An enormous papillary overgrowth has led to formation of thick masses of mucin in which many disintegrating cells are included. The mucin becomes red in the muci-carmine stain. Under these intestine-like glands other ducts are seen with low cuboidal clear epithelium and non-mucinous hyaline-like contents; they look similar to kidney tubules and they are situated in renal blastema as one sees it in embryonal kidney tumors of children. There are many abortive tubules and irregular small agglomerations of cells similar to the epithelium of the tubules. No striated muscle found. The ureter is lined by what appears as mucosa of large intestine. No renal blastema seen in the ureter.

This tumor cannot be classified together with any reported so far. It belongs halfway between the embryonal adenomyosarcoma of kidney and the intestine-like more or less tumorous changes which develop in the depth of the everted urinary bladder. Its similarity to large intestine is still more marked. It is a hamartoblastoma, probably multicentric or even diffuse in pelvis of kidney and ureter. Considering the rarity of such tumors and the frequency of kidney stones one cannot ascribe to the stone an important role in the genesis of this tumor. Embryonal kidney tumors have been observed repeatedly in the adult. The prognosis is doubtful especially because ureter with intestine-like epithelium has remained in the patient.

Discussion

(Dr. E. T. Bell, Minneapolis.) Out of 100 renal tumors from adults I have found two adenosarcomas of the infantile type, but these were unlike Dr. Plaut's cases in that they did not show mucin. The presence of mucin is difficult to explain, and in this respect these tumors differ from adenosarcomas previously described.

(Dr. Plaut, closing.) As to the presence of mucin I would like to make the general statement that I think the importance of mucin has been over-estimated. Often glandular structures coming from peritoneum might form mucin. I have seen mucin form in Fallopian tubes. I might add that there was no gelatinous transformation of connective tissue. Connective tissue was widely destroyed by gelatinous material.

SKELETAL METASTASES IN MALIGNANT TUMORS OF THE KIDNEY. Isaac Levin, New York City.

(*Abstract not received.*)

STUDY OF ARTERIES IN NON-SUPPURATIVE DISEASES OF THE KIDNEYS. N. W. Barker (by invitation), Rochester, Minn.

Abstract. The celluloid corrosion method has been used in a study of the course, relations, distribution and patency of the arterial system in human kidneys. A demonstration will be given of the gross arterial trees and photomicrographs of their terminal branches in some cases of non-suppurative renal disease as compared with those of normal kidneys.

Discussion

(Dr. Alfred Plaut, New York City.) How soon after removal of the organ or after death were these kidneys injected? Was the tissue still alive at the time of injection? Contraction of the blood vessels under the influence of the injection might change the picture.

(Dr. Barker, closing.) Of course it is impossible to obtain the kidney as you would like to have it. It is not possible to show functional changes in vessels. We obtain them as soon as we can and at postmortem they are washed out with ordinary tap water. Formerly normal saline was used. I believe it makes very little difference in the postmortem changes in the kidney. I think the kidneys were stained at least three hours after death, sometimes longer, five or six hours. All normal tissue, under normal conditions, shows no variation.

THE ARTERIAL SUPPLY OF THE KIDNEY IN NEPHRITIS AND ITS RELATION TO THE CLINICAL PICTURES. Saul A. Ritter (by invitation) and George Baehr, New York City.

Abstract. 1. Extensive vascular alterations similar to those of primary arteriolar disease develop as a regular phenomenon in cases of chronic diffuse nephritis (chronic Bright's disease) which survive the glomerulonephritis for a long enough period, and are not an accidental complication.

2. It is not alone the primary diffuse parenchymal disease, as has been generally thought, but rather this secondary vascular alteration which is responsible for the sclerosis and contraction of the secondary contracted kidney. Primary and secondary contracted kidneys in this respect are pathogenetically identical.

3. The clinical picture of arterial hypertension terminating finally in dry uremia may be associated with either the primary or the secondary contracted kidney. In our experience, the secondary contracted kidney is in fact much more commonly found at postmortem as the cause of this picture.

Injection studies upon the renal arterial system explain one of the apparent discrepancies between clinical findings and pathological types of chronic nephritis, and demonstrate that complete harmony exists if the significance of the essential pathological process is recognized.

NEPHROSIS WITH GLOMERULONEPHRITIS (CASE REPORT). Margaret Warwick, St. Paul, Minn.

Abstract. A case report of a woman 34 years of age who had scarlet fever at the age of 18 and diphtheria at the age of 32. Her present illness was of six months duration and consisted of malaise, drowsiness, edema, ascites and high blood pressure, with casts, albumin and low specific gravity in the urine, and a secondary anemia. Her creatinin went up to 7.2 and urea nitrogen up to 47, but her eye-grounds remained normal.

The autopsy showed a complete absence of the left kidney, although a small, patent ureter and pelvis were present. The right kidney weighed 300 gm., the capsule was loose, the cortex thick and pale, but the pelvis was normal. Microscopic sections showed lesions affecting both the glomeruli and the tubules. In some of the glomeruli there was a proliferation with the formation of new connective tissue and the fusion of the tuft with Bowman's capsule; in many others there was a marked infiltration with polymorphonuclears and a swelling of the endothelium of the tuft capillaries, while yet others remained normal in appearance. The tubules all showed a marked atrophy consisting of a decrease in size and a lining of very low or flat epithelium. The tubular injury seemed to be out of all proportion to the glomerular injury since there were some normal glomeruli, and no normal tubules could be found. Furthermore, the lesions in many of the glomeruli do not seem to be severe enough to account for the extensive tubular atrophy. Therefore, lesions seem to be affecting the tubules and glomeruli independently instead of the tubular injury being secondary to the glomerular injury. The only possible classification seems to be the so-called mixed form with the presence of both a nephritis and a nephrosis.

Discussion

(Dr. B. H. Lucké, Philadelphia.) It seems to me that all these cases present the usual picture of subacute glomerulonephritis.

(Dr. G. Baehr, New York City.) I would like to agree with the previous speaker and call attention to the fact that the clinical diagnosis of nephrosis is

being made altogether too frequently. Every case of glomerulonephritis has a certain nephrotic element. At the Mt. Sinai Hospital in New York, where we have studied nephrosis for a long time, we have only once encountered a pure nephrosis in an adult at autopsy. Every other case clinically diagnosed nephrosis has at autopsy presented characteristic evidences of glomerulonephritis. The one time nephrosis occurred in an adult was in a syphilitic. In children we do see pure nephrosis not infrequently. In my experience they are usually superimposed upon a chronic pneumococcus infection in the upper respiratory tract. Eventually most of these children die of a pneumococcus infection of their ascites.

(Dr. K. M. Lynch, Charleston, S. C.) It seems to me that there are two separate lesions in the kidney. I agree with the other speakers that the term nephrosis is not applicable. It seems to me there is an older interstitial sclerosis and an apparently recent glomerulonephritis. The dilatation of tubules may be related to the absence of the other kidney. Not uncommonly I see true chronic diffuse interstitial nephritis. I see no reason why this condition with superimposed subacute glomerulonephritis could not explain this kidney.

(Dr. Warwick, closing.) I feel justified in the case report because of the discussion brought out. I think the discussion of the term "nephrosis" has been very well answered. Perhaps my title on the program was not clear, I think it should not have been stated as it was. I meant to say I had lesions of both tubules and glomeruli, so I called it nephrosis with glomerulonephritis, which is not as accurate as it might be. With these kidneys we feel very definitely that the lesions of the tubules were out of proportion to the lesions in the glomeruli and the absence of the other kidney may be the cause of it.

EXPERIMENTAL GLOMERULONEPHRITIS PRODUCED BY THE TUBERCLE BACILLUS.

Leone McGregor (by invitation), Rochester, Minn.

Abstract. Calmette's attenuated bovine tubercle bacillus (BCG) was introduced intra-arterially into the kidneys of rabbits.

The injections were made into the superior mesenteric artery against the blood stream, after first applying two clamps, one on the aorta below the left renal artery, the other on the superior mesenteric artery just distal to the point of injection. In this way most of the BCG emulsion was driven through the renal arteries. The kidneys of 10 rabbits were removed at regular intervals so that stages from one to twenty days could be studied.

Sections of these kidneys show exudative and marked proliferative changes in a large proportion of the glomeruli, with obliteration of their capillary circulation and beginning tubular atrophy.

Discussion

(Dr. E. R. Long, Chicago.) This is of special interest to me in connection with the studies I have just made myself, to be reported in the next paper. I would like to ask Dr. Bell if more than one injection was made. If so, the phenomenon of sensitization might have entered. It is interesting to recall that the tubercle bacillus is a rather complex structure in which more than one kind of chemical substance is present and important. There are the proteins, to which the host becomes specifically sensitive, and there are the lipoids which act as a foreign body. It would be interesting, therefore, to know if there was any difference between injecting the whole bacterial cell and injecting simply the lipoid. I have made intracardiac injections, but never produced as severe a picture as

that shown and did not reach anything like sixty per cent of the glomeruli. I have been accustomed to interpret the changes occurring in the tubules when the glomeruli are blocked off as atrophy from anemia and therefore malnutrition, rather than atrophy from disuse. Is not that the case?

(Dr. G. Baehr, New York City.) Years ago we attempted to produce glomerular lesions by injection of streptococci into the renal artery and found it impossible to produce bacterial emboli in any very large percentage of the glomeruli. The reason is obvious since Richards has demonstrated that all of the glomeruli are not working all the time. You therefore cannot expect by any intravascular injection to damage all the capillaries in all the glomeruli or in any very large percentage of them.

(Dr. Long.) Do you think that would be the case if you made a perfusion with a special apparatus which might be run over half an hour? All the glomeruli are not in use at one time. There are cycles, a particular group carrying the load for 10 to 15 minutes, and then closing for a period. Do you not suppose you might get all the glomeruli if the injection were properly prolonged?

(Dr. E. T. Bell, Minneapolis.) The needle was inserted into the superior mesenteric artery and two injections were given with an interval of about 15 minutes. It is not desirable to occlude all the glomeruli since this results in uremia. We have used only Calmette's attenuated bovine culture, BCG. This is an ideal organism to produce proliferative lesions.

EXPERIMENTAL SUBACUTE GLOMERULONEPHRITIS PRODUCED BY INTRARENAL TUBERCULIN REACTIONS. Esmond R. Long and (by invitation) Lucy Finner and Paul J. Patchen, Chicago, Ill.

Abstract. Acute and subacute forms of glomerulonephritis were produced by arterial perfusion of the kidneys of tuberculous swine under ether anesthesia with a finely flocculent suspension of purified tuberculin protein. Similar perfusion of the kidneys of normal swine resulted in no appreciable change. The exudation within the glomeruli was therefore interpreted as a true tuberculin reaction, analogous to that produced by the injection of tuberculin into the skin or testis of a tuberculous animal. Epithelial and endothelial organization of the glomerular exudate resulted within two months in glomerular changes similar to those seen in some forms of subacute glomerulonephritis in man. In the course of ten months after the operation almost complete recovery occurred, only a small amount of glomerular scarring persisting. The course of anatomical change was observed by biopsy, nephrectomy or killing of some of the animals in the series. During the subacute stage of the process, after bilateral perfusion, the non-protein and urea nitrogen of the blood were more than doubled. With anatomical recovery the blood chemistry returned to normal.

Discussion

(Dr. P. O'Hare, Boston.) I would like to ask the speaker if he feels that this is a sensitization process. If it is such, why does he not get the same reaction with the tuberculin alone?

(Dr. C. W. Duval, New Orleans.) I would like to ask Dr. Long and his coworkers if they found it necessary to make the injections of tuberculin directly into the renal vessels; could not the same lesions be produced by giving the tuberculin subcutaneously or intracardially?

(Dr. E. T. Bell, Minneapolis.) Dr. Long has shown us a very good example of focal glomerulonephritis. This is evidently a localized tuberculin reaction in

the kidney and corresponds to the skin reaction in a sensitive animal. The question is whether or not this sensitization plays a rôle in human nephritis.

(Dr. S. R. Haythorn, Pittsburgh.) Even though we can get a glomerulonephritis experimentally in this way it is difficult to see what happens in the tuberculous subject. Unless the kidney is able to protect itself in some way every tuberculous patient should develop a glomerulonephritis each time a caseating lesion releases tuberculous toxin. All tuberculous patients then, should die early of glomerulonephritis. It is well known that tuberculous cattle may be given a desensitizing dose of tuberculin which will protect them from a positve tuberculin test for several weeks. This procedure is known as "plugging." A similar mechanism may apply in the tuberculous patient since one rarely finds acute glomerular lesions. A few years ago I reported a case of a tuberculous aneurysm of the common iliac artery and in the glomeruli of the kidney there were masses of tubercle bacilli. Most of the glomeruli showed no reactions. There were capillary loops blocked with tubercle bacilli in which no reaction of any kind was present. If a tuberculin reaction occurs in a sensitized kidney in the human it must be a very temporary thing or else the kidney must protect itself in some way, perhaps by "plugging" as the expression is applied to cattle.

(Dr. G. Baehr, New York City.) The rôle which sensitization plays in the production of human glomerulonephritis is best brought out possibly in subacute bacterial endocarditis due to the streptococcus viridans. In our observations about 17 per cent of patients with subacute streptococcus endocarditis develop a complicating acute diffuse glomerulonephritis. This acute glomerulonephritis has no relation to the embolic glomerular lesions found in this disease. The true acute glomerulonephritis seems to occur only in those patients who have shown a tendency to kill off their bacteria. With few exceptions the patients with subacute bacterial endocarditis who died of an acute glomerulonephritis or its sequel, chronic diffuse nephritis, were all in the bacteria-free stage of the disease as described by Libman, or were becoming bacteria-free. Patients with subacute streptococcus endocarditis, in whom the blood cultures are persistently positive, and who show no such tendency to become bacteria-free, do not as a rule develop an intercurrent diffuse glomerulonephritis.

(Dr. Long, closing.) In reply to Dr. Duval's question, as to whether subcutaneous injections would not work just as well as intrarenal injections, the answer is no. We found it necessary to make these into the renal artery. Subcutaneous and even intravenous injections were not sufficient.

With regard to Dr. Bell's remarks: I feel myself that probably the lesions one sees in the kidneys in subacute bacterial endocarditis may be partly on this allergic basis. It is certainly probable that if a patient has this disease for some months the body becomes sensitized. The interaction between the sensitized tissue of the glomeruli and the sensitizing bacteria might well result in what we call subacute bacterial nephritis.

I am greatly interested in the work of Dr. Johnson, having developed the medium in which the bacteria for the work were grown. Colleagues associated with him have worked chiefly with the injection of these products, similar to the one we used here, in normal animals and also in normal cells, such as the ameba, and I believe they are dealing with a special type of foreign body reaction to substances differing in their individual primary toxicity. It is not the type of reaction we are concerned with here, which has to do with the reaction between specifically sensitized cells, and the antigen to which they are sensitive.

I think the answer to Dr. Ewing's question is that this work may not be so far removed from application to the problems in which we are interested as he supposes. There are other reactions taking place in the body, reactions we are all familiar with, that might be interpreted as tuberculin reactions. For instance, a tuberculous process spreads from the lung of a person infected with tuberculosis to the pleura; the result is an acute pleuritis, pleuritis with effusion, which many people believe is really a tuberculin reaction. Joint lesions in tuberculosis and tuberculous meningitis may likewise be truly responses of sensitized tissues to the substance to which they are sensitive, with the added element of morphological tuberculosis, of course, in all these instances.

There are probably many non-specific changes taking place in tuberculosis also. I admit it is still doubtful whether the kidney shares in this type of reaction. I cannot reconcile these results entirely with the facts to which Dr. Haythorn has called attention. A closer study of the facts is needed. To Dr. Baehr's question I might answer yes. We use a suspension in order to delay the speed of progress through the kidney. We have to put the reaction partly on an embolic basis, lasting a few minutes or more likely several hours.

MITOCHONDRIA IN EXPERIMENTAL ACUTE NEPHRITIS. Robert A. Moore (by invitation), Columbus, Ohio.

Abstract. An attempt has been made to determine the variations that mitochondria undergo in bichloride nephrosis and to correlate these findings with the degree of functional impairment of the cells involved. The mitochondria were best studied with the anilin fuchsin-methyl green stain (Cowdry's modification) but iron hematoxylin and Janus green B were also used. White rats were used and blood urea determinations were made on all animals just prior to death. After carefully eliminating all normal variations the following conclusions were reached. The mitochondria in the third part of the convoluted tubule are the first to show any variation. This variation comes after there has been renal insufficiency as evidenced by urea retention. In other words there is renal insufficiency before there are changes in the mitochondria.

These changes are a slight swelling and marked agglutination. Later the same changes take place in part 1 and 2 of the proximal convoluted tubules. On the basis of these findings, it is proposed that bichloride produces a nephropathy first by being adsorbed at the mitochondrial-cytoplasmic interface and later because of increasing concentration at this point the entire cell is killed.

EXPERIMENTAL STUDY OF ACQUIRED RESISTANCE OF THE RABBIT'S RENAL EPITHELIUM TO URANYL NITRATE. Warren C. Hunter (by invitation), Portland, Oregon.

Abstract. Although the ability of renal epithelium to regenerate after injury has been frequently demonstrated, little is known concerning the nature of the new cells. The purpose of this study was to determine whether regenerated kidney cells are as susceptible to injury by nephrotoxins as the original epithelium or become resistant or immune to such substances. The experimental work of Gil y Gil, the only previous investigator in this field, has been repeated and confirmed.

Preliminary experiments showed that 0.001 gm. of uranium subcutaneously or 0.0005 gm. intravenously, each representing one-half the lethal dose for rabbits, regularly kills normal proximal convoluted tubular epithelium. Complete regeneration occurs in two weeks. Differences in morphology and staining

readily distinguish the new epithelium from the original. Ample time for regeneration was allowed between the periodically increased injections. Only those animals surviving the last injection sufficiently long for fresh necrosis to appear in the persisting original tubular cells and less than the time required for new regeneration to begin are included in the series.

A high degree of acquired resistance to uranium is evidenced by lack of microscopic changes in the regenerated epithelium after giving 54 to 96 times the amount known to kill the original cells in the same tubules. Intravital staining indicated that the regenerated tubules were patent. Qualitative chemical tests and biological experiments proved that chronic uranium kidneys were able to excrete the metal in greater quantities than acutely damaged organs. The acquired resistance may be due either to the cells detoxicating the poison or to preventing its combination with their proteins.

In rabbits long-continued repeated administration of uranium leads to marked chronic glomerulo-tubular lesions with secondary contraction.

CORRELATED GROSS, MICROSCOPIC AND CLINICAL OBSERVATIONS UPON 160 CONSECUTIVE PAIRS OF KIDNEYS. James E. Davis, Ann Arbor, Mich.

Abstract. Material: A series of 200 consecutive autopsied subjects who had been patients in four of Detroit's hospitals.

Plan of Study: The routine clinical records represent standard data obtained from all types of cases entered in the hospital as house patients. Eighty-five per cent of the total number were patients in the Receiving Hospital of the City of Detroit, and fifteen per cent were from Providence, St. Mary's, and Woman's Hospitals. It should be mentioned that a certain number of emergency cases included had insufficient data.

In addition to the general survey made at each autopsy by three members of the staff in Pathology and the writers, special routine studies were made of the kidneys, observations being directed to:

1. The thickness and adherence of the capsule.
2. The appearance of the cortex after the capsule had been stripped.
3. The relative proportions of the cortex and medulla seen on section.
4. The amount of peripelvic fat.
5. The presence or absence of striations in cortex and medulla.
6. The amount of apical blanching in the pyramids, and its degree.
7. The character of the pelvic mucosa.
8. The character of the superior part of the ureters.
9. The total weight of the organs stripped of fat.

Microscopic observations were made of two to four sections from each case, and attention was directed to changes in the arterioles, arteries, tubules, stroma and mucosa. In addition to diagnosing the presence of infection, degeneration, or both, an estimation was made of the degree of postmortem autolysis; also whether or not the blood pressure was raised; whether there was nitrogen retention, albuminuria, edema or drainage insufficiency. These observations were made without any knowledge of the clinical or gross conditions, and gradings were made by a second person who held the known clinical and gross data. This plan was used in order to evaluate, independently, the microscopic changes. The results obtained are of considerable value in proving the utility of certain routine observations and a classification, based quite strictly upon anatomical divisions of the kidney.

It was found advantageous to observe arteriolar changes in the glomeruli and their afferent and efferent vessels as the chief criterion of rise in blood pressure and nitrogen retention. Degeneration of the tubular units after excluding the effects of postmortem autolysis was used to indicate the effects of toxicity and infection. Stromal tissue increase was considered a factor decreasing efficiency in drainage. Albuminuria was diagnosed where active degeneration was present, and where active inflammatory reaction prevailed, accompanied by marked arteriolar reaction. Our practical experience proved that estimation of the presence of edema was most difficult. It is reasonable to expect this because the condition of the kidney is not solely responsible for the production of edema. However, correct estimation of the condition was made in sixty-five per cent of the cases.

If the additional information obtained from the gross study of the kidney removed as a surgical procedure be considered with the microscopic information, the correlation work will be enhanced. It is to be remembered that the microscopic and gross evaluations, when viewed solely from the clinical standpoint, may be considered at first thought impractical. However, it may be emphasized, that accurate and painstakingly acquired clinical data will point as surely to fixed structural changes, as will structural changes to the clinical diagnostic essentials.

The following classification was found most effective for correlation:

Vascular (arterio and arteriolar) nephritis and nephrosis
Tubular nephritis and nephrosis
Stromal nephritis and nephrosis
Focal nephritis (and focal pyonephritis) and nephrosis
Ureteronephritis and pyoureteronephritis
Perinephritis.

GENERAL DISCUSSION OF GLOMERULONEPHRITIS, NEPHROSIS AND RENAL ARTERIOSCLEROSIS. E. T. Bell, Minneapolis, Minn. (Appointed by the Council to act as reviewer).

Abstract. Classification of Nephropathies:

I. Nephroses
   (*a*) Chemical
   (*b*) Infectious
   (*c*) Pregnancy
   (*d*) Lipoid
   (*e*) Amyloid
II. Glomerulonephritis
   (*a*) Focal
      1. Embolic
      2. Benign hemorrhagic
   (*b*) Diffuse
      1. Acute
      2. Subacute
      3. Chronic
         (a) With lipoid nephrosis
III. Arteriosclerotic Kidney
   (*a*) Without renal insufficiency
   (*b*) With renal insufficiency
      1. Slowly developing type
      2. Acute fulminating type

IV. Exudative Interstitial Nephritis
  (*a*) Acute interstitial nephritis
  (*b*) Pyelonephritis
    1. Hematogenous
    2. Urinogenous

In classifying the nephropathies, pathologists should keep in mind the functional as well as the structural changes in the kidneys. In so far as possible, we should adjust our nomenclature to correspond with recognizable clinical entities. An ideal classification based entirely on etiology is not possible at present, since the same organisms (*viz.*, streptococci and pneumococci) seem to cause certain forms of nephrosis as well as glomerulonephritis and exudative interstitial nephritis. The symptoms and clinical course of a nephropathy are determined by the following circumstances: (*a*) the histologic elements affected, *i.e.*, glomeruli, tubules, arteries, interstitial tissues; (*b*) the *character* of the process, *i. e.*, exudative, proliferative, degenerative; and (*c*) the *degree* of the injury. The foregoing classification is based partly upon the histologic elements affected and partly upon the character of the lesion.

I. Nephroses

Nephropathies of a purely degenerative character are called nephroses. The lesions are chiefly confined to the tubules but often the glomeruli and rarely the arterioles show degenerative changes. Glomerular lesions of a degenerative character are classified as nephroses. The alterative changes are cloudy swelling and fatty metamorphosis in the milder forms and hyaline granular degeneration and necrosis in the severe types.

(*a*) *Chemical Nephroses.* The representative of this group which is of greatest clinical interest is the corrosive sublimate kidney. This is characterized clinically by oliguria and anuria, moderate albuminuria, slight edema and progressive renal insufficiency. Associated with the anuric stage, there is often a moderate hypertension. Anatomically there is marked tubular degeneration and necrosis, often with calcification of the necrotic tubules.

The experimental nephropathies obtained with uranium nitrate, potassium bichromate, and so forth, belong with the nephroses, since except for an occasional epithelial crescent, the only glomerular lesions obtained are of alterative character. This is true even of the experiments of Wiesel and Hess, who obtained extensive necrosis of the glomeruli. Purely alterative glomerular lesions should not be called glomerulonephritis.

(*b*) *Infectious Nephroses.* Nearly all severe infectious diseases or infections are accompanied by albuminuria of varying degree; and at postmortem in such cases the kidneys show cloudy swelling. In rare instances, there is anuria clinically, and a widespread necrosis of tubules and glomeruli is found at postmortem. Necrotic nephroses of this type have been described in diphtheria, cholera and severe septicemia.

The infectious nephroses show gradual transitions to acute glomerulonephritis. There is often a slight swelling of the glomerular endothelium, and when this change becomes sufficiently pronounced it is called glomerulonephritis. Often the decision between infectious nephrosis and acute glomerulonephritis is arbitrary. The pathogenesis of glomerulonephritis is best studied in the infectious nephroses.

The infectious nephroses are rarely cases of renal disease in the clinical sense, since they are definitely overshadowed by the major illness.

(*c*) *Nephrosis of Pregnancy.* The toxemia of the latter part of pregnancy (called eclampsia when convulsions occur) is characterized by albuminuria, hypertension and edema. There is often oliguria and there may be anuria; but usually renal function is fair. At postmortem the kidneys are found cloudy and greatly swollen. There is a moderate amount of lipoid in the tubules and glomeruli. Occasionally there is moderate or even extensive tubular necrosis. In five of ten cases which I have studied microscopically, there was a marked swelling of the glomerular endothelium with definite narrowing of the capillaries. The enlargement of the glomeruli is only slight and I have not proved that proliferation occurs; but the effect on the circulation is the same as that of a glomerulitis.

Aside from the glomerular changes, which are not constant, the anatomic picture corresponds with that of a lipoid nephrosis. A few chemical studies that have been made do not show clearly whether the chemical changes in the blood correspond with those observed in pure lipoid nephrosis.

(*d*) *Lipoid Nephrosis.* This is the same as the "genuine nephrosis" of Volhard and Fahr, the "chronic nephrosis" of Epstein and the "lipoid nephrosis" of Munk. Pure cases of lipoid nephrosis are characterized clinically by severe albuminuria and edema, and the absence of hypertension and renal insufficiency. There is an increase of blood cholesterol and a decrease of the plasma proteins, especially of the albumin, so that the albumin-globulin ratio becomes reversed. Lipoids are found in the urine and the edema fluids are low in protein. It is a chronic disease that usually ends fatally but sometimes in recovery.

Certain cases of chronic glomerulonephritis show edema, albuminuria, increased blood cholesterol and decreased serum proteins. These may be distinguished from a pure lipoid nephrosis whenever hypertension or renal insufficiency is present; but since both of these signs are occasionally absent in glomerulonephritis, we must admit that the final diagnosis of pure lipoid nephrosis must be made postmortem. Notwithstanding the difficulty in establishing the diagnosis clinically, there can be no doubt that cases of pure lipoid nephrosis do occur.

The kidneys are large and cloudy and there is abundant lipoid in the tubules. The glomeruli are normal.

We have no definite information as to the etiology of pure lipoid nephroses. It is known that the partially obstructed glomeruli of chronic glomerulonephritis are often associated with a large tubule full of lipoid; but there is no anatomic glomerular damage in pure lipoid nephrosis.

(*e*) *Amyloid Nephrosis.* This renal lesion is usually secondary to chronic suppuration and is overshadowed clinically by the infection. There is usually marked albuminuria, and edema is frequent. Sometimes the blood shows the changes characteristic of a lipoid nephrosis. The anatomic changes are amyloid infiltration of glomeruli with hyaline, granular and fatty degeneration of the tubules. The glomeruli often become completely occluded so that the tubules atrophy and a contracted kidney results. Cases that end with renal insufficiency often show hypertension also. One case of amyloid kidney which I studied developed acutely following a streptococcic suppuration and in association with an acute glomerulonephritis. The amyloid kidney is related to glomerulonephritis.

II. Glomerulonephritis

(*a*) *Focal Glomerulonephritis.* When only a part of the glomeruli show inflammation, the lesion is called focal glomerulonephritis.

1. *Embolic Glomerulonephritis.* A well recognized nephritis of this type is Löhlein's embolic form, which develops in association with bacterial endocarditis. Clinically, it appears as a complication of bacterial endocarditis and is often recognizable by the appearance of hematuria in this form of cardiac disease. Microscopically, the typical lesions are areas of necrosis involving one or more lobules in a glomerulus. These lesions are believed to result from small infected emboli from vegetations on the valves of the heart. Occasionally lesions of a proliferative character are noted which involve a part or all of the glomerulus. Glomerular abscesses are occasionally observed. Diffuse glomerulonephritis is almost as frequent as the embolic type as a complication of bacterial endocarditis.

2. *Benign Hemorrhagic nephritis.* In children and young adults, one often sees hematuria which lasts from a few days to several weeks. The hematuria follows some infection such as a tonsillitis and is not accompanied by edema, hypertension or renal insufficiency. Fahr calls this condition focal non-embolic or excretory glomerulonephritis; but Baehr's suggestion, "benign hemorrhagic nephritis" seems to be more descriptive of the disease. No pathologic studies are available, but a study of hematurias associated with fatal infections suggests that the underlying anatomic change is rupture of the wall of glomerular capillaries. It is possible that the disease is a very mild diffuse glomerulonephritis, since many cases of hemorrhagic nephritis are known to be of this type. When anatomic studies become available it may be necessary to put this disease in some other group.

(*b*) *Diffuse Glomerulonephritis.* In well developed cases of this disease, nearly all the glomeruli show inflammatory changes. Clinically the characteristic features are albuminuria with casts and often with blood, edema and hypertension. Edema varies greatly in different cases and also from time to time in the same individual. Hypertension may be absent in mild acute cases and in certain chronic cases.

The glomerular lesions are of three histologic types: exudative, proliferative and extracapillary.

(1) The exudative lesion consists in the accumulation of large numbers of polymorphonuclear leucocytes within the glomerular capillaries. The leucocytes may pass out into the capsular space and into the tubules. Pure exudative glomerulitis is uncommon; there is usually an associated proliferation of the endothelium.

(2) Proliferative glomerulitis is the most common type. It consists in swelling and proliferation of the glomerular endothelium with enlargement of the glomerulus and narrowing or closure of the capillaries. Polymorphonuclear leucocytes are often imprisoned in the closed capillaries.

(3) The extracapillary type consists in proliferation of the cells of the parietal layer of the capsular space with the formation of an epithelial crescent which compresses the glomerulus.

Pure exudative lesions probably cause only temporary occlusion of the glomerular capillaries, but proliferative lesions cause permanent narrowing or complete closure. When the blood can no longer go through the glomerulus, secretion of urine ceases and the tubule undergoes disuse atrophy. By the time that the glomerulus has become hyaline, the associated tubule has disappeared.

Atrophied tubules are replaced to some extent by connective tissue — an appearance which is responsible for the term "chronic interstitial nephritis" which is often applied to the contracted kidney of chronic glomerulonephritis.

When the glomerular capillaries are narrowed but not completely closed, the associated tubule does not atrophy but enlarges, and the cells accumulate lipoid droplets. The large kidneys of this type were formerly called "chronic parenchymatous nephritis." This form of chronic glomerulonephritis is characterized by severe albuminuria and edema and often shows the changes in the blood characteristic of lipoid nephrosis. Hypertension is sometimes absent.

III. Arteriosclerotic Kidney

(*a*) *Without Renal Insufficiency.* Approximately 90 per cent of persons who have primary hypertension do not show renal insufficiency. The kidneys show varying degrees of involvement of the arteries and arterioles but there is not sufficient atrophy to cause a definite decrease of renal function. The parenchyma does not undergo atrophy until the artery or arteriole is markedly narrowed. There are all degrees of atrophy in the kidneys of persons who die of primary hypertension, from normal kidneys to small contracted kidneys. Only about 10 per cent show a sufficient degree of atrophy to justify the term "primary contracted kidney." In hypertension without renal insufficiency, death is due to congestive heart failure, coronary sclerosis or apoplexy.

(*b*) *With Renal Insufficiency.*

1. The usual case of this group shows clinically a slowly developing uremia. The kidneys are small and show granular surfaces — "primary contracted kidney." The atrophy is due to narrowing and closure of both small arteries and arterioles.

2. Certain persons who have had hypertension for a long time without any signs of serious renal involvement develop uremia rather acutely, often as a result of some infection. Occasionally, an acute hypertension with uremia develops in a person who was previously normal. Such cases are sometimes called "malignant" hypertension. The kidneys in these cases may not be contracted, but microscopically a widespread necrosis of arterioles is found.

IV. Exudative Interstitial Nephritis

(Not discussed at meeting)

The Histopathology and Bacteriology of Experimental Poliomyelitis in the Monkey. E. C. Rosenow, Rochester, Minn.

Abstract. Cultures of brain and cord of 142 monkeys that developed poliomyelitis following inoculation of virus, by methods similar to those previously described, yielded greening streptococci in ninety-two or 65 per cent, indifferent streptococci in 8 per cent, hemolytic streptococci in 6 per cent, staphylococci in 46 per cent and bacilli in 25 per cent. Greening streptococci were isolated in 80 per cent of eighty-seven monkeys that were cultured in from one to three days after onset of paralysis, in 60 per cent of twenty cultured four to six days after onset of paralysis, in 37 per cent of sixteen cultured seven to ten days after onset of paralysis, and in 26 per cent of nineteen cultured eleven days or longer after onset of paralysis.

Similar cultures from the central nervous system of fifty-six monkeys inoculated intracerebrally with the greening streptococcus yielded this organism in

52 per cent, indifferent streptococci in 9 per cent, hemolytic streptococci in 12 per cent, staphylococci in 27 per cent and bacilli in 21 per cent. Greening streptococci were isolated in 60 per cent of fifteen monkeys cultured from one to three days after inoculation, 69 per cent of sixteen monkeys cultured four to six days after inoculation, 25 per cent of eight cultured from seven to ten days after inoculation and 41 per cent of seventeen cultured eleven days or longer after inoculation. Cultures from sixty-nine control monkeys, chiefly those that remained well following inoculations of virus and of the streptococcus and those that died of tuberculosis or colitis, yielded the greening streptococcus in 25 per cent, indifferent streptococci in 9 per cent, hemolytic steptococci in 4 per cent, staphylococci in 26 per cent and bacilli in 10 per cent. The agglutinating power of various batches of the poliomyelitis antistreptococcus serum over greening streptococci was tested in order to determine their immunologic condition. Ninety-two per cent of seventy-nine strains isolated from seventy-nine monkeys that developed poliomyelitis following inoculations of virus were agglutinated specifically by this serum. Ninety-two per cent of thirteen strains isolated from thirteen monkeys inoculated with the streptococcus obtained in cases of epidemic encephalitis and epidemic hiccup were agglutinated specifically in contrast to 13 per cent of fifteen strains isolated from fifteen control monkeys.

A microscopic study of sections of brain and cord was made of thirty monkeys that had acute poliomyelitis following inoculations of the virus and of twenty-five controls, mainly those that remained free or had doubtful symptoms following inoculations of virus, and those examined a long time after onset of symptoms and in which sections were free from poliomyelitic lesions. Of the former, all had typical lesions of poliomyelitis in varying degree. By the use of a modified Gram method of staining and diligent search, Gram-positive cocci and diplococci of varying size and shape, sometimes arranged in short chains and clumps of large and exceedingly small forms, were found in or adjacent to lesions in each of the thirty monkeys that had symptoms and lesions of poliomyelitis. In some instances similar organisms were found in direct smears of the cerebral and ventricular fluid and crushed material from the anterior horn of medulla and cord. The series included animals inoculated intracerebrally and intraspinously with fresh and glycerolated emulsions of virus and filtered virus as well as animals that developed poliomyelitis following intravenous injection and after packing of the nose with gauze soaked in emulsions of virus. Diplococci were uniformly absent in sections and smears of control monkeys as well as in the normal tissues of the poliomyelitic animals remote from the lesions.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) May I ask a question from the depth of ignorance? In the first table it seems there were 85 per cent organisms other than the streptococcus viridans in the first set of animals and in the control set of animals with streptococcus viridans and not only were there no organisms recovered, but there was 69 per cent of other organisms. It would be interesting to know what Dr. Rosenow thinks the reason for their being there and also whether that does not change the etiological significance of the streptococcus viridans to a certain extent.

(Dr. Rosenow.) I did not quite get Dr. Krumbhaar's point. You say there were other organisms?

(Dr. Krumbhaar.) Yes.

(Dr. Rosenow.) You were adding all other types.

(Dr. Krumbhaar.) Are you not allowed to add them?

(Dr. Rosenow, closing.) Yes, you may add them but it would have no meaning. The exact significance of the isolation of the species of bacteria other than the greening streptococcus is not entirely clear. However, in view of the fact that considerable exposure to the air is unavoidable in obtaining pieces of brain and cord and in emulsifying these for culture, that the highest percentage consisted of staphylococci and bacilli, that so often they were found in only one or two of a large number of tubes inoculated, that they were isolated nearly as often in the control series as in poliomyelitic animals, and that these and the indifferent and hemolytic streptococci were not agglutinated by the serum of affected animals nor by the poliomyelitis hyperimmune horse serum, there is little doubt but that they were chiefly contaminations from air or passive invaders. Since greening streptococci were isolated in a far greater percentage of animals than any other species and in proportion to the earliness in the attack and since this is the only species that had antigenic effects as measured by the agglutinating power of the serum of inoculated animals, the isolation of other organisms in smaller numbers, over which the serum showed no agglutinating power, need not detract from the etiologic significance of the greening streptococcus.

STATISTICAL STUDY OF THE CAUSES OF DEATH IN 200 CASES OF COARCTATION OF THE AORTA (ADULT TYPE). Maude E. Abbott, Montreal, Canada.

Abstract. The findings in a personal case of complete obliteration of descending arch with impending rupture of the ascending aorta and with cerebral death in a boy of 14, are compared with 199 other cases in subjects over two years, in the literature, and a statistical study is presented showing the degree and character of the stenosis, relative frequency of a collateral circulation, cardiac hypertrophy, bicuspid aortic valve, acquired valvular lesions and acute infectious hypoplasia or dilatation of the aorta and of the various modes of death, in these 200 cases. The material reviewed consists of 136 cases abstracted by certain older writers on this subject, and 64 collected from the recent literature, the latter with the help of Dr. L. Minor Blackford of the Mayo Clinic.

Coarctation is commoner in the male than in the female sex, the proportion in this series being as 3 to 1, or 147 males to 48 females. Its symptomatology usually reveals itself in athletic muscular subjects in early adult life, after the myocardium has become weakened by infection or unusual physical strain; and death commonly occurs in the prime of middle life, either abruptly from arterial rupture, or as a result of failing compensation in the presence of acute infections or their cardiac sequelae. Among these 200 cases, 148 (74 per cent) died before or during the fortieth year of life, so that, in spite of its rarity, this condition is one of great clinical and medico-legal importance. This fact is not sufficiently appreciated at the present time.

So-called "spontaneous" rupture of the heart or aorta (usually by way of a dissecting aneurysm in two stages) had taken place in 40 cases, in two of which the seat of rupture was in the right wall of the heart, in 33 in the ascending aorta, and in 5 at the seat of coarctation. The ascending aorta was dilated in practically all the cases of rupture in this situation and microscopic examination of its wall (made in 13 of the cases) usually showing medial degeneration, while a congenitally bicuspid valve was present in over 50 per cent of these 33 cases and in 22 per cent of the entire series. From these facts and the wide dissection of the tear a congenital weakness of the aortic media is suggested.

Infective endarteritis and mycotic aneurysm of the aorta took place in 14 cases, in 13 at the seat of coarctation, and in one immediately above the aortic valve; in the latter case death had taken place from rupture of the mycotic aneurysm, as also in 3 at the seat of coarctation. This makes a total of 44 cases of rupture of the aorta among the 200.

A serious neurological lesion was present in 26 cases, and in 20 of these it was cerebral hemorrhage. In 2 of these the hemorrhage was due to septic embolism, but in the other 18 it was "spontaneous" in origin, and in 5 of these the presence of a ruptured cerebral aneurysm was demonstrated at autopsy. In the remaining 13 cases the youth of the subjects, the symptoms of intermittent leakage, and the entire absence of any known cause, suggests that these also were due to rupture of a small congenital aneurysm, the result of a primary weakness of the wall of these arterioles of congenital origin.

THE RELATION OF INTRAVASCULAR PRESSURE TO ARTERIOSCLEROSIS. Eli Moschcowitz, New York City.

Abstract. In the study of the etiology of arteriosclerosis one must take into consideration not merely a study of arteriosclerosis of the greater circulation but of the pulmonary circulation as well. Furthermore, a sclerosis comparable to the lesion of arteriosclerosis also occurs in the veins and especially the capillaries. Arteriosclerosis of the pulmonary circulation is independent of arteriosclerosis of the greater circulation, and only occurs when a condition causing increased pressure of the pulmonary circuit is present, such as mitral stenosis, emphysema, infiltrative lung lesions, open ductus Botalli or communications between the right and left heart. The pathology of the capillary lesions in pulmonary arteriosclerosis was discussed and the similarity of these to the glomerular lesions in hypertension of the greater circulation. The question of primary sclerosis of the pulmonary vessels — Does it occur? Decrescent arteriosclerosis of the greater circulation associated with long-continued normal intravascular pressure begins early in young adults. Decrescent arteriosclerosis of the pulmonary vessels occurs at a much later age, because the normal pulmonary pressure is one-sixth that in the aorta. The independence in incidence of arteriosclerosis between the greater and lesser circulations excludes obviously such causes as infections, metabolic products, etc.

The relation of experimental cholesterol deposits in the vessels to the problem of the etiology of arteriosclerosis was discussed, especially their similarity to the lipoid deposits in the intima seen in infants on a high fat diet and in lipemia. These experimental lipoid intimal infiltrations are probably not "arteriosclerosis." The subject of arteriosclerosis of the lower animals was reviewed, and the universality of arteriosclerosis in all peoples and in all ages. Arteriosclerosis occurs in those portions of the vessel that are fixed or have firm support. The only common factor in all arteriosclerosis is normal or increased intravascular pressure.

THE CIRCULATION OF BLOOD THROUGH THE SPLEEN PULP. Ward J. MacNeal, New York City.

Abstract. The splenic lobule consists of the malpighian corpuscle or follicle, its surrounding marginal zone and the irregularly radiating pulp cords surrounding this, together with the included vascular channels. Its periphery is marked by a zone of larger venous sinuses which serve adjacent lobules.

The intralobular or follicular arteriole is a terminal branch of the splenic artery without anastomosis with its fellows. From it there arise very abundant, extremely delicate vessels which form a capillary plexus within the follicle. These follicular capillaries communicate with each other. Their walls consist of a single continuous layer of delicate endothelium which retains the erythrocytes within the lumen but evidently permits the escape of fluid. In microscopic preparations most of these vessels are collapsed and appear as solid cords of endothelial cells. It appears probable that the passage of blood through them is intermittent in the living spleen. At the margin of the follicle these delicate capillaries terminate in the pulp at a considerable distance from any venous capillary. The endothelial cells of the wall become continuous with the reticular cells of the marginal zone and the lumen, if patent, opens into the intercellular spaces of the pulp. Sometimes a dilatation or rather poorly defined ampulla is recognizable at the termination.

Somewhat coarser branches of the follicular arteriole pass directly out of the follicle and curve about in the red pulp. These capillaries may give off several branches. They possess peculiarly thickened walls for part of their course, such a thickening constituting the ellipsoid sheath of Schweigger-Seidel. Some of the terminal twigs curve back toward the follicle as the centripetal capillaries of the marginal zone. The wall suddenly becomes reduced to a single sheet of endothelium and then the vessel opens out into the pulp spaces of the marginal zone, sometimes dividing and becoming distended before terminating.

Rather longer capillaries originate from the same trunk and extend along the pulp cords toward the periphery of the lobule, where they terminate in asymmetric ill-defined ampullae situated within the substance of the pulp cord. These ampullae open into the intercellular spaces of the pulp.

The intercellular spaces of the pulp open into the venous capillaries through the stomata of Mollier, which are present everywhere between the rod cells and the circular fibrils which form the walls of these capillaries. These vessels form a richly anastomosing plexus, running between the pulp cords from the marginal zone to the periphery of the lobule, where they pass over into coarser venous channels lined by a continuous sheet of endothelium and thence to the trabecular veins.

THE VENOUS DRAINAGE OF THE SPLEEN. W. L. Robinson, Toronto, Ont.

Abstract. The generally accepted conception of the structure of the venous sinuses of the spleen is that presented by Mollier (*Arch. f. Mikr. Anat.*, 1910–11, lxvi, 608). He described most minutely the variations in their structure and the general principles underlying them as seen in mammals and humans. One would gather from his article that the venous sinuses were a continuation of the arterial system, for he says, "The veins do not form an independent system but are merely canals formed in the pulp tissue." He points out that they are bounded either by the unchanged pulp tissue or in specially developed forms, *e. g.*, the human, with a protoplasmic syncytial lattice made up of longitudinal bands united by transverse bridges and supported by a reticulum; a perforated structure having stomata of various sizes and shapes. These apertures in the walls of the veins allow for the passage of the blood plasma and cellular elements from vein to pulp or from pulp to vein.

Using a Zeiss Binocular microscope with stereoscopic attachments, a study of the distended cat spleen reveals the fact that the venous system in the cat is

not continuous with the arterial system but is independent. The larger veins are enclosed wholly or in part by the trabeculae. The smaller branches are very short and made up of syncytial sheets of endothelial protoplasm which open out in a bell-shaped manner to communicate directly with the pulp spaces. The lining syncytium of the terminal veins blends almost imperceptibly with the pulp cells. There are a few rounded stomata in the walls of these terminal veins, but the main inlets are through the bell-shaped openings at their origins. The venous sinuses of the dog and human spleens are a little different from those of the cat in that their sinuses are much longer, more numerous, and have myriads of slit-like stomata in their walls. We have not as yet been able to demonstrate the beginnings of the individual venous branches in the dog and human spleens.

The findings in the cat spleen would seem to be further evidence of the independence of the arterial and venous capillary systems and positive proof of an open circulation for the spleen.

Discussion

(Dr. W. C. MacCarty, Rochester, Minn.) I am not clear in my own mind. He says the blood goes into the pulp from the arterioles, thence into the venous capillaries. I would like to know what the sinuses are. Maybe you made that clear but it was not clear to me.

(Dr. W. J. MacNeal, New York City.) It is true that if one has studied the perfused spleen it is possible to see in ordinary sections of the spleen more detail than before. I can say that is true in my own case. At the same time I do believe the routine perfusion of the spleen is something that is worth while and such technique as we have described does not interfere with the usual ordinary preparations. If you can get tissue two hours after death there is no reason why you cannot perfuse necropsy material. If you cut off a portion of the spleen from one end for ordinary fixation and smears and then clamp that end you can then go ahead and perfuse the remainder. The pulp of the spleen cannot be well fixed after four hours because the cells undergo lysis.

The discharge of blood from the blood vessels into the intercellular spaces of the pulp occurs most abundantly in the marginal zone around the follicles and I believe that the small capillaries within the follicle normally allow no red blood cells to pass through their walls but do allow the plasma to pass through. The concentrated corpuscles remain within the lumen to be discharged into the pulp spaces around the follicle. I regard this as a mechanism by which the fluid portion of the blood is brought into relation with the lymphocytes of the follicle where it may be acted upon chemically. The concentrated corpuscles are thrown into intercullular spaces of the marginal zone where they come into immediate relationship with the pulp cells and may be phagocyted or allowed to pass through if they are in good condition. Normally, the plasma and the corpuscles after passing this inspection join again in the pulp spaces and get back into the venous circulation through the stomata of Mollier.

As fast as we can work in the injection of particulate matter in suspension followed by perfusion fixation we find that the formed particles injected are always in the marginal zones. This material also escapes into the pulp cords but the amount here is much less. The marginal zone is evidently a most important part of the splenic lobule.

In reply to Dr. MacCarty's question as to the sinuses: I avoided the use of the term except perhaps through a slip of the tongue, because it may be misunderstood. In general the term sinuses designates the venous spaces and vessels of

the spleen which possess fenestrated walls composed of rod-shaped endothelial cells. The term does not apply to the intercellular spaces of the pulp.

(Dr. Robinson, closing.) Nothing more than to concur with Dr. MacNeal. We have made a number of India ink injections of the spleen. We found that if minimum amounts were injected intravenously it was filtered out by the ellipsoids. If larger quantities were injected it first appeared in the pulp proper about the malpighian corpuscles. I agree with Dr. MacNeal that the red blood cells pass on out through the end capillaries which are permeable, into the pulp spaces.

RETICULUM IN THE LUNG IN POST-INFLUENZAL ORGANIZATION. G. R. Callender, Washington, D. C.

Abstract. The organizing processes in the exudate in alveoli, air sacs, atria and bronchioles in the pneumonic processes following the 1918 pandemic of influenza were studied to determine the sequence of events leading to the final organization into fibrous connective tissue. The steps in order were found to be as follows:

1. Increase in large mononuclear cells (histiocytes) in the walls of atria and alveoli and in the exudate. The latter was usually granular with little fibrin, rarely fibrinous, occasionally purulent.
2. Extension of reticulum fibers from the reticulum of the walls into the exudate.
3. Formation of collagen by fibroblasts in the exudate.
4. Disappearance of reticulum as the collagen increased but without evidence that reticulum was transformed into collagen.

Delicate fibers of both collagen and reticulum were present in the same area in the exudate. Because of this finding collagen fibers are considered to differ chemically from reticulum fibers.

Discussion

(Dr. N. C. Foot, Cincinnati.) We shall never get the answer as to the difference between reticulum and collagen by means of staining methods alone; we can never decide as to whether they are chemically different until we approach the question from the chemical standpoint. I believe that we have exhausted the resources and utility of staining methods in this connection; we can get just so far and then we are up against a barrier. For that reason I have been tackling the subject from the standpoint of chemical differences, or chemistry. If you take a small piece of spleen and boil it in water for four hours, you will find that you are unable to stain it with acid fuchsin, the staining properties of the collagen are lost. Furthermore, if you evaporate this aqueous extract down to a syrup, you can recover the collagen in the extract; in other words, what we call "collagen" (from the microscopical standpoint) is apparently a material soluble in hot water. It is not gelatinous. On the other hand, if you boil spleen in water the reticulum does not dissolve. If you boil it in alcohol the reticulum will be more or less affected and dispersed through the tissue. Digestion with pancreatin destroys all of the cellular material in a spleen, leaving the connective tissue undigested; if you dissolve this in weak alkali and neutralize the solution, you will get a precipitate in which you can (with the silver carbonate-fuchsin method) stain material that looks very much like reticulum. There will be typical fibrils in this material. On the other hand, if you wash this precipitate, there will be nothing much that will stain with acid fuchsin. We are appar-

ently dealing with two substances that are closely related and I believe that, in the case of reticulum, collagen may be associated with some other substance that gives this tissue its characteristic argyrophilic tendencies.

If you use the silver carbonate method on formalin-fixed material, you may or may not get a reticulum impregnation, but if you will first subject this tissue to the Mallory method of oxidation and reduction (potassium permanganate followed by oxalic acid) you are almost certain to get 100 per cent impregnation of the reticulum. If, on the other hand, you treat such tissues with reducing agents, such as pyrogallol or sodium sulphite, you can entirely inhibit the impregnation of reticulum. All this has no effect upon the collagen whatsoever. So it seems to me that we are gradually accumulating evidence that there is a chemical difference between these substances.

Dr. Mallory brought out, last year, a statement that this is merely a mechanical difference, the compact fibrils (which are loosely arranged) responding to silver and the more diffuse fibrils that are felted together in fibrous masses not being permeable to the silver solution and therefore not impregnating. I think that, if this were true, we should get a silver mirror over the surface of the sections after silver treatment, which is not the case.

I submit these points for your consideration.

(Dr. E. B. Krumbhaar, Philadelphia.) I ask a question again for information. Did not Dr. Mallory satisfactorily show last year as regards the histogenesis, that both reticulum and collagen could come from fibroblasts?

(Dr. Callender, closing.) I want to thank Dr. Foot for his discussion. In regard to Dr. Krumbhaar's question. It is my understanding that what Dr. Mallory said last year was that collagen and reticulum were one and the same and it depended upon the size of the fiber whether it would take up the reticulum or collagen stain. Therefore, it was implied that they both arise from the same cell, namely fibroblasts.

A reticulum cell is indistinguishable to me from a young fibroblast, but there will be reticulum in one place and in another collagen.

METADYSENTERY, WITH REMARKS ON CERTAIN GROUPS OF INTESTINAL BACTERIA. Aldo Castellani (by invitation), New Orleans, La.

Abstract. Some years ago I introduced the following classification of bacterial dysenteries:

1. Bacterial dysentery *sensu stricto*, due to the Shiga-Kruse bacillus (does not ferment lactose, does not ferment mannitol).

2. Paradysentery, due to Flexner, and Flexner-like bacilli including Hiss-Russell (does not ferment lactose, ferments mannitol).

3. Metadysentery, due to the "metadysentery bacilli." The "metadysentery bacilli" are organisms which like the true dysentery bacilli (Shiga, Flexner and Flexner-like or dysentery-paradysentery organisms) do not produce gas in any sugar, but in contrast to the true dysentery bacilli they produce acidity to litmus in lactose peptone water and clot milk, or produce acidity in lactose without clotting in milk or clot milk without producing distinct acidity to litmus in lactose peptone water.

These bacilli were grouped by Chalmers and myself into two genera dysenteroides and lankoides. The organisms of the dysenteroides group do not clot milk, those of the lankoides group clot milk. In practice, however, it is of advantage to combine the two genera into one (*dysenteroides sensulato*) with the general characters I have mentioned.

Principal types of the metadysentery bacilli

The following bacilli may be considered to belong to the metadysentery group:

1. *Bacillus pyogenes foetidus* Passet 1902. This and similar organisms although belonging culturally to the metadysentery group do not seem to cause dysentery or any other clinical type of colitis. They were found in abscesses.

2. The bacilli found by me in dysentery and certain peculiar types of colitis febrile and afebrile, which I described with the names *B. ceylonensis A*; *B. ceylonensis B*; *B. metadysentericus*. In addition *B. gintottensis* and *B. madampensis*.

3. Some strains discovered by Duval in his important investigation on the etiology of infantile diarrhoea seem to me to belong to the metadysentery group rather than to the paradysentery group or Flexner group.

4. Certain strains found in England in cases of infantile diarrhoea by Nabarro who, however, until recently identified them with *B. coli anaerogenes*.

5. Certain strains found recently by Sonne in cases of dysentery unless these strains be identical with *B. ceylonensis A*; *B. ceylonensis B*; and *B. metadysenterious*.

The pathogenic metadysentery bacillary may be separated into two principal types. (1) *Bacillus ceylonensis A* type (including *B. metadysentericus*); (2) *Bacillus ceylonensis B* type. In the first type acidity in lactose peptone water is produced very slowly and clotting of milk may be absent. Indol is usually negative. In the second type acidity in lactose is produced rapidly and milk is clotted also fairly rapidly. Indol is usually positive.

There are several different types of metadysentery. In this paper I have called attention to a chronic type which is not of rare occurrence. In this type true dysenteric symptoms, *viz.*, blood and pus in the stool are often lacking. The patient feels tired, nervous, depressed, has no appetite, complains of vague abdominal discomfort, dyspepsia, flatulency, with occasional attacks of diarrhoea often followed by long periods of constipation. A similar clinical syndrome may be caused by *Entamoeba histolytica* but rarely by bacilli of the Shiga and Flexner type.

I shall be pleased to supply workers interested in the subject with cultures of the strains of metadysentery bacilli which I have in my possession.

THE BIOLOGICAL ACTION OF RADIANT ENERGY. I. ULTRAVIOLET. Eugene R. Whitmore, Washington, D. C.

(*Abstract not received.*)

STUDIES ON LIPOCHROMES. Charles L. Connor, Boston, Mass.

Abstract. This report deals with several aspects of the question, the first of which had to do with the reaction of animals to carotin. This, the principal lipochrome found in the animal body, was extracted from carrots and purified. Upon injection intraperitoneally into guinea pigs granulomatous lesions are formed similar to familiar foreign body reactions. It does not appear in the blood or urine of guinea pigs after injection or ingestion of relatively large amounts. It produced no effect in a rabbit when injected intravenously.

By the use of fat stains on frozen sections of carrots, adrenal gland, corpus luteum, and chicken skin, and on tissues removed from animals after injection of cholesterol and other lipoids, and by the use of other reagents which might be expected to distinguish lipochromes, an effort was made to differentiate carotin, xanthophyll, cholesterol, and other lipoids from one another. It was found that

lipochromes do not stain characteristically with fat stains; that Nile blue sulfate does not differentiate cholesterol from other lipoids except neutral fat; but that, by its double refractiveness, lipochrome is most often associated with cholesterol or an ester of cholesterol. Lipochrome is easily bleached with $FeCl_3$, is soluble in $CHCl_3$ after dehydration with acetone, and crystallizes after treatment with alcoholic KOH and formalin. It cannot be seen normally as a granular substance in any tissue which contains it except the outer layers of the skin, because of its fine dispersion in its fatty solvent.

Lipochrome was found by chemical or histologic methods to be present in the skin, fat, liver, spleen, adrenal cortex, and corpus luteum. Ten methods, chemical and histologic, including those found to be useful in identifying carotin, and the dopa reaction, silver nitrate, basic fuchsin, and iron stains, were employed to differentiate the other common pigments. Hemofuscin and hemosiderin were found to be present together in the heart, intestinal muscle, seminal vesicles, testicles, prostate, and in old hemorrhagic foci. These two pigments are increased in brown atrophy of the heart and liver. Melanin seems to be confined to nervous tissue or ectodermal cells. Two pigments, therefore, increase in amount in advancing age, namely, lipochrome, which is exogenous and comparatively unimportant, and hemofuscin with an iron-containing pigment, which may be derived from muscle hemoglobin.

ABERRANT THYROID GLANDS. Lawrence W. Smith and (by invitation) John V. Leech, Boston, Mass.

Abstract. Four cases of lateral aberrant thyroid tumors are presented with the clinical and pathological findings. Their probable etiology from the so-called fifth pharyngeal pouch is discussed. The possible relationship of the papilliferous tumors of the thyroid gland itself to these same cell rests, after migration, is raised.

SARCOMAS OF THE LIVER. Ernest Scott and (by invitation) Harry L. Reinhart, Columbus, Ohio.

Abstract. The paper is concerned with the presentation of an unusual case of multiple primary tumors of the liver, arising in the reticulo-endothelial system of that organ.

The tumor is of a polymorphous character, and the microscopic sections reveal areas in which there is a marked proliferation of reticulo-endothelial cells, with destruction of liver parenchyma, or formation of an endothelial syncytium. Other areas are sarcomatous with diffuse hemorrhages scattered throughout the substance.

TUMORS AND MALFORMATIONS OF THE BLOOD VESSELS OF THE CENTRAL NERVOUS SYSTEM. Percival Bailey (by invitation), Boston, Mass.

Abstract. Tumors of the cerebral blood vessels may be divided essentially into vascular malformations (telangiectases, angioma venosum, angioma arteriale) and hemangioblastomas. The former are composed of vessels (capillary, venous or arterial) separated by neuroglia. The latter are true neoplasms composed of angioblasts which may form either capillary spaces, solid masses of endothelial cells or cavernous spaces filled with blood or serum. No neuroglia is found within these tumors but an abundant reticulum is present. The hemangioblastomas are most common in the cerebellum and are often cystic. They are often associated with angiomatosis of the retina and sometimes with cystic pancreas,

cystic kidneys, hypernephromata, etc., in a pathological complex now known as Lindau's disease.

Examples of the different types of these tumors will be described with the aid of lantern slides.

A CONTRIBUTION TO THE PATHOLOGY AND ETIOLOGY OF MEXICAN TYPHUS. H. Mooser (by invitation), Mexico City, Mexico.

Abstract. Male guinea pigs inoculated with Mexican typhus show swelling and inflammation of the scrotum. Histological examination reveals that the primary lesion takes place within the endothelial lining of the tunica vaginalis. Stained smears from the proliferated tunica show swollen endothelial cells containing a minute diplo-bacillus which multiplies so abundantly that the cells finally rupture. This liberation of the microörganism is followed by the local reaction and fever. The infected cells exhibit a striking resemblance to the stomach cells of lice containing *Rickettsia prowazeki*. The tunica is highly infectious as is evidenced by inoculation into guinea pigs and the accidental infection of a laboratory worker with typhus.

Discussion

(Dr. Lillie, Washington.) I have had an opportunity in the past year to compare the histological tissues of typhus from Dr. Wolbach's laboratory and from the one isolated by Dr. Maxcy. I think we can fully confirm Dr. Connor's statements of the entire absence of the scrotal lesions, whereas we find the same thing that Dr. Mooser described in Mexican typhus. I had occasion to look at a section of Dr. Mooser's this morning and some of my own and I would hate to tell which is which. I have one photographic negative which I took last evening which shows the similarity of what we have to what Dr. Mooser described.

In the brains of the cases which we were studying we were able to see typical nodes in about one-half the guinea pigs which I think is intermediate between what Dr. Mooser finds and the European strain. I might also say in spotted fever I have not found them in the few animals I have examined.

(Dr. Mooser.) Dr. Wolbach was in Mexico in 1919 and published his first report on Mexican typhus and he did not find any difference. I did not see any difference in Mexican typhus and European typhus except that the rash in Mexican typhus was paler than European typhus, beginning like measles and becoming hemorrhagic later. The brain lesions are much more rare in Mexican typhus in human beings than in guinea pigs and we were always able to find them in guinea pigs. I might observe that the scrotal swelling was probably responsible for the rarity of the lesions. The female will show many lesions in the gray matter of the brain. The male shows very few lesions. The testicle protects the brain from being involved. The lesion in the vascular endothelium is always most pronounced.

(Dr. C. L. Connor, Boston.) The lesion which has been described and the picture which I have seen is extremely like that of the lesion of Rocky Mountain fever. Ricketts, in 1908, called attention to the similarity between typhus fever and Rocky Mountain spotted fever as far as clinical lesions and the external lesions produced in humans are concerned. No one has ever described before such a lesion in the guinea pig in true typhus fever or typhus fever in Europe, and in the strain which has been carried on in the Harvard Medical School no such lesion has ever appeared, although these animals have been injected intraperitoneally. I think the route of injection has nothing to do with it. On the

other hand, these lesions occur in Rocky Mountain spotted fever although they are injected subcutaneously or in some other way. It has been demonstrated that whereas spotted fever is carried by a particular tick in the spotted fever region, it may also be carried by any other tick; so that the fact that typhus and Mexican typhus, which seemed to be the same until now, may not be carried by the same thing is not surprising. It is most important to find out what the carrier is; whether it may not be a tick and whether it is not more closely related to Rocky Mountain fever than European typhus.

(Dr. Maxcy, Washington.) I think Dr. Mooser has made a very important contribution to our knowledge of typhus. If Rickettsia-like bodies can be demonstrated consistently and convincingly in guinea pigs experimentally infected with typhus virus, then the strongest argument against the etiologic relationship of Rickettsia to typhus will have broken down.

We are more interested, however, in the possibility that the pathologic lesion which Dr. Mooser describes may be used to differentiate North American typhus from Old World typhus. Dr. M. H. Neill of the U. S. Public Health Service first described swelling and redness of the scrotum of guinea pigs inoculated with a strain of typhus isolated on the Mexican border. Dr. Mooser has confirmed and extended this observation so that its specific character seems established. I have found the same lesion present in guinea pigs inoculated with the endemic typhus of the southern United States, which by the way is not conveyed by lice. In other respects these guinea pigs reacted as do guinea pigs inoculated with Old World typhus. Dr. Lillie has made a detailed study of the microscopic pathology and in the main his observations confirm Mooser. The pathology of guinea pigs inoculated with Mexican typhus and with the endemic typhus of the United States appears to be similar, and to present certain characteristics which are different from those usually manifested in guinea pigs inoculated with Old World typhus.

(Dr. A. Plaut, New York City.) Can one really compare the nodules which were shown outside the infarcted area with nodules in the tissue? I never saw nodules in typhus in any organ, including the central nervous system, which did not chiefly consist of histiocytes, together with some plasma cells, occasional leucocytes, etc. These nodules on serial sections always were found connected with a small blood vessel. I wonder if these loosely lying masses which were shown can be compared with the typhus nodule.

(Dr. Allen, Charlotte, N. C.) In dealing with endemic typhus in the south it is simple to convey it directly to guinea pigs. We have not been able to demonstrate in the brain the pathology described by Dr. Wolbach but have noticed constantly the orchitis demonstrated this morning. Neither lice nor ticks have anything to do with our endemic typhus.

THE EARLY DIAGNOSIS OF MALIGNANCY. W. C. MacCarty, Rochester, Minn.
(*Abstract not received.*)

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